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REMARKS ON POLLINATION BY BATS IN THE GENERA FREYCINETIA, DUABANGA AND HAPLOPHRAGMA, AND ON CHIROPTEROPHILY IN GENERAL

BY

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This paper contains a collection of notes on chiropterophily. They form part of the material I intend to include in a general review of the subject, which will be published in book-form. As it may take some time before this work appears, it seemed worth while to issue a selection of this material beforehand.

Recently a concise review, stressing general points, has been published in French by JAEGER (1954). It includes some new observations on *Ceiba* and *Adansonia* made by Jaeger himself.

1. FREYCINETIA (*Pandanaceae*)

It seems to me a commendable procedure to start these notes with some remarks on the genus in which pollination by bats was first observed and described.

BURCK (1892) described in a work on the Botanical Gardens at Buitenzorg how bats feed on the juicy bracts by which the inflorescences of a *Freycinetia* are surrounded, but do not damage the flowers. He thought it likely that the bats effect pollination, and suggested that the plant might be dependent upon these animals for its survival. The bats that were responsible for this feat were held to be flying foxes (*Pteropus*), but the choice of his words suggests that this was a mere guess.

A still earlier observation made by Moseley and quoted by KNUTH-LOEW (1904) as possibly referring to *Freycinetia*, cannot apply to this genus, as the plant Moseley had in view, is said to be a tree, whereas *Freycinetia* is a liana.

During his stay in Java in 1899 Knuth observed in the Botanical Gardens at Buitenzorg, as described in KNUTH-LOEW (1904), bats visiting the "flowers" of a *Freycinetia* species, for which he uses the name *Fr. strobilacea* Bl. The bats observed by him, were not the large flying fox, but a small and a medium-sized species, which he thought might be resp. *Pteropus minimus* Geoff. and *Cynopterus marginatus* Geoff. He regarded both as fructivorous species, but we now know that the first (syn. *Macroglossus minimus*) feeds on nectar, and does not have the power to dissect the bracts.

Knuth noted that the juicy inner bracts and the erect „*Beköstigungskörper*" (food-bodies), which are metamorphosed bracts inserted between the spadices, serve as food for the bats.

For a long time the genus was passed on in the literature as the standard example of pollination by bats.

Porsch (1915) doubted its chiropterophily, and correctly stated that the *Freycinetia* species which dominates in the famous *Canarium* avenue at Buitenzorg, is ornithophilous. He drew the attention to its diurnal flowering, its bright red colour and its lack of smell. It was for him the first instance of a flower provided with food-bodies that is pollinated by birds. According to him the bats were mere plunderers.

In a later paper Porsch (1923) identified the species which he had studied as *Fr. funicularis* (Rumph) = *Fr. funicularis* Merr. He says that the plants were in 1914 labelled as *Fr. strobilacea* Bl., and that this was the reason why he had used this name in his first, preliminary publication. Porsch described in detail how the flowers are pollinated by the bird *Pycnonotus aurigaster* Vieill., and suggested that incidentally pollination may be brought about by a fructivorous bat.

Since that time the observations made by Burck and Knuth have fallen into discredit, although Porsch (1935) maintained the genus in his list of plants in which bat visits have been observed. Jaeger (1954), however, absolutely denied the occurrence of chiropterophily in the genus.

Do we really have to discredit Burck's observations? Certainly not! In other fields of study I could already confirm some of the contested ideas of this former sub-director of the Gardens, who doubtless made mistakes, but who certainly deserves to be honoured as a pioneer in the field of tropical ecology.

When we carefully examine what he actually says on p. 67, we will see that he does not mention a species by name, and it will be clear that the plant to which he referred, was not *Fr. funicularis*, but probably *Fr. insignis* Bl., for he describes the flowers as pinkish. We may assume, therefore, that a specimen of this species grew in 1890 in the garden. At the present time *Fr. insignis* still occurs in the neighbourhood. A coloured plate is given by Blume (Rumphia I, tab. 42).

With regard to the description given by Knuth, I can only say that Knuth and Loew possibly confused the two species. Their fig. 3 agrees with *Fr. funicularis*.

Fr. insignis is undoubtedly chiropterophilous.

In a paper which appeared in 1941 I mentioned that I intended to publish an article on this matter, but during the war and revolution in Java my manuscripts and notes were lost. In August 1950 I could study the plant once more in a forest (alt. 1400 m) near Bandung.

On inflorescences that had flowered in the preceding night, I found in the morning on the hard outer bracts marks of bat claws. The erect white food-bodies and most of the pinkish or pale-lilac inner bracts had been removed. Of the more peripheral bracts, which are provided with harder tips, only the basal part had been bitten away. (c.f. fig. 1 and 2). The parts that were left clearly showed the imprints

PLATE I

Fig. 1. Freycinetia insignis Bl. from above. Male inflorescence in the morning following anthesis. Edible bracts consumed by bats. Top of half-edible bract spared.

Fig. 2. Freycinetia insignis Bl. The same inflorescence as in Fig. 1 from the side; some of the enveloping bracts and one spadix removed.

Fig. 3. Drooping branch of Duabanga moluccana Bl. with terminal flower cluster. Late evening.

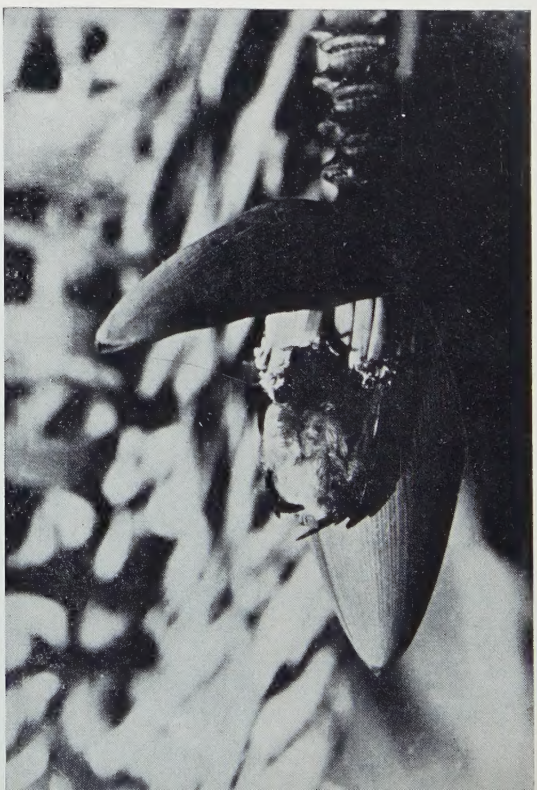

Fig. 4. Flowerstand of Musa paradisiaca with visiting Macroglossus. Late evening.

made by the teeth of the nightly visitors, which could in this way be identified as belonging to *Cynopterus*, a well-known genus of fruit-eating bats. From the Indonesians living in the neighbourhood we heard that fruit-bats were during the night regularly fighting round the inflorescences.

The spadices themselves are apparently unedible. I found no imprints of teeth on them.

At 13.— p.m. I took some inflorescences home. They were on the verge of opening but emitted as yet no fragrance.

At home I could make photographs and observe the process of anthesis. It is probable that it was accelerated by the transport in the dark luggage hold of the car.

Early in the afternoon the outer bracts began to separate, exposing the edible inner bracts. Pollen was set free and a sweet fruit odour, but mixed with a musty component, began to escape. Late in the evening the opening was completed and the odour became much stronger and also more pronouncedly musty.

It is possible that the inflorescences retain their attractiveness for a second night, although during the latter the female spadices become discoloured.

The pinkish to pale-lilac colour agrees with that observed in other bat-flowers, e.g. those of *Dombeya*. The position of the flowers, at the end of branches that stand away from the stem of the supporting tree, causes the free exposure that is so typical of flowers that are pollinated by bats.

The taste of the food-bodies and bracts is at first sweet, but afterwards it becomes disagreeable to us, viz. wry as of tannic acid.

As always when studying a flower belonging to a certain ecological class, we find that it fits the senses of the legitimate visitors and excludes others. Once the legitimate visitors know the place, they can often find flowers that are missing one of the usual signals. Fructivorous bats also take unripe fruits that emit as yet no smell. This happens also in *F. insignis*, where some inflorescences are robbed by bats the night before anthesis. This may also explain why bats may eventually shift their attention to the inflorescence of a neighbouring *F. funicularis* which emits no smell at all.

F. insignis does not belong to the large group of chiropterophilous plants (which consists of at least 25 genera), which offer nectar and pollen to strongly specialized bats that feed on nectar, such as *Macroglossus*. It belongs to the small group of plants, formed by *Madhuca*, *Bassia* (*Illipe*), and possibly *Pachira insignis*, which offer solid foodtissue to the less specialized fruit-bats, which occasionally may deviate from their ordinary line of fruit-eating, subs. flower-squashing.

In the class of flowers, that are pollinated by beetles, the use of solid tissues is often regarded as a primitive character, but this does not necessarily hold true for the chiropterophilous flowers just mentioned. They seem to follow a new line, parallel to that of the main group. I find as yet no reason to assume in the *Pandanaceae* former connections with beetles or primitive vertebrates.

It is possible that other Javanese *Freycinetia* species are chiropterophilous, as for some of them smelling flowers are mentioned.

It might also be of interest to study the pollination of the New-Zealand *F. Banksii* A. Cunn., of which the white or pale-lilac bracts are eaten by man. This happens also with the bracts of *F. marquisensis* and of *F. monticola* which occur in the Marquesas. The bracts of the latter are orange or red.

In 1927 HEIDE published observations made in Bogor (Buitenzorg) on *F. funicularis*. It is rather remarkable that he saw no nocturnal visits of bats, and said nothing on diurnal visits of birds. He observed squirrels (*Sciurus notatus* Bodd), visiting and destroying inflorescences in the afternoon. He supposed that these animals might incidentally cause pollination.

A relation to rodents (rats) seems lately to have been established in the Hawaiian *F. arborea* Gaud. The rats are not indigenous to the islands, and the inflorescences, though they spread an odour, look as if they might be ornithophilous. Observers have reported visits by native birds.

Sinclair reported already in 1885 the activity of rats on the bracts of this species, and DEGENER (1945), says that the inflorescences are provided with fragrant, bright orange, fleshy and sweet tasting bracts, which are eaten by rats, but he does not mention what happens to the spadices. The whiskers and fur of the rodent proved to be covered with pollen. As the plants fruit well, the visits seem to lead to regular pollination.

2. DUABANGA MOLUCCANA BL. (*Sonneratiaceae*)

The tree is indigenous in the Moluccas, but a specimen is cultivated in the Botanical Gardens Bogor (Buitenzorg). In 1941 I made observations on this tree, but the notes were lost during the war and revolution. The relationship with the chiropterophilous *Sonneratia acida* led to the discovery.

I do not remember whether actual visits of bats were observed, but I do remember that both species were at the time included in a list of plants that were proven to be chiropterophilous.

The flowers open at night, are creamy-white, spread a strong, sourish-sweet odour and produce the abundance of nectar that is typical of this class. The anthers are uncommonly large. The position of the flowers is also typical, for they are found at the end of drooping branches, well exposed and easily accessible. Here again the structure of the tree proves to be more or less adapted to bat-visits. The stem and main branches emit long horizontal side branches. The latter are rather far apart and their ends are drooping (c.f. fig. 3).

3. HAPLOPHRAGMA ADENOPHYLLUM (WALL.) DOP. (*Bignoniaceae*)

In my study "Fledermäuse und Blumen" (1936) I already pointed at the frequent occurrence of chiropterophily in the family *Bignoniaceae*,

especially in the *Markhamia—Dolichandrone—Heterophragma* group, to which the above-named species belongs (syn. *Heterophragma adenophyllum* Seem. ex Benth. et Hook., *Spathodea adenophylla* D.C.).

The species is indigenous to Birma and Malaya and has large, fleshy flowers of a dull-brown-yellow colour. They spread a typical bat-odour —as I could observe in May 1949 in a specimen cultivated in the Botanical Gardens in Bogor. The swellings on the lower lobes of the corolla make it easier for the bats to obtain a foothold.

The flowers are nocturnal and in the morning, when they are shed, they show the typical marks of bat claws.

The shape of the tree is identical to that of the chiropterophilous *Markhamia stipulata*. This means that it is of the "pincushion type" with the flowers borne on long, stiff stalks which project on all sides of the crown—a typical chiropterophilous position.

4. Adansonia Gregorii F. v. Muell (*Bombacaceae*)

This Australian species is, like its better known relative from Africa, chiropterophilous.

I could observe a specimen in Bogor (Buitenzorg) in June 1952. The yellowish-green flowers open in the night and are shed in the morning, showing at that time the claw marks of bats.

5. Comment on new examples recently recorded by other authors

a) **Ipomoea albivenia**

The communication by Vogel (1954) deserves to be cited here. He mentions as characteristics of the flower: broadly campanulate shape, a nearly white colour set off with a dull-violet throat, nocturnal anthesis and a disagreeable odour reminding one of garden swedes. He thinks that the chiropterophily is questionable, as he found no traces of bat visits. He was, moreover, not sure whether flower-visiting bats occur in South-Africa.

As, however, the finding place (Zoutpansbergen) lies in or near the tropical region, either *Megaloglossus*, or the fructivorous *Eidolon*, which according to Jaeger (1954) has developed a taste for nectar, might be present. Vogel referred to another Convolvulacea, viz. *Erycibe ramiflora*, which I should have regarded as chiropterophilous, but I wish to point out that I (in 1936) merely suggested that it might be so.

b) **Eugenia cauliflora**

Porsch (1941) thought it probable that this species was to be placed in the group of chiropterophilous plants provided with solid food-bodies for attracting visitors (from which group he omitted *Madhuca*). I think that the prophet and grandmaster of chiropterophily here too has correctly understood the chiropterophilous character. One point, which he does not mention, though it is easily discernable in

his photographs, is the large size of the anthers, which far surpasses that of its sister-species. For parallel cases in other genera I may point to *Bauhinia megalandra*, to *Duabanga moluccana*, to *Eperua falcata* and to the chiropterophilous Cactaceae (c.f. PORSCH, 1939).

This point, however, fits in (like the increase in the number of anthers elsewhere) with the character-complex of those chiropterophilous plants which offer nectar and pollen as food. It might be, therefore, that the flower does not belong to the group to which it has been assigned by Porsch, and that in its natural habitat it produces nectar. Fleshy petals are found in the main group also.

c) **Mucuna monosperma**

As the short note on this flower by BÜNNING (1952) is hidden between considerations of a different nature, I wish to draw the attention to it.

Whereas most *Mucuna* species are flagelliflorous, this one is cauliflorous. The photographs clearly show claw marks, identical with the bat marks described and figured by me for *M. reticulata* (1941). This find too shows the relation existing between cauliflory, flagelliflory and bats.

6. PLANTS THAT HAVE ERRONEOUSLY BEEN REPORTED AS CHIROPTEROPHILOUS

I refer here in the first place to the list given by PORSCH (1935). Porsch himself stated that some cases were dubious. Unfortunately this list is sometimes used uncritically.

a) **Piper aduncum**

The original statement by HEIDE (1927) was based on an oral communication by an observer who merely said that bats similar to those caught on *Kigelia* visited this plant, but this observer did not differentiate between fruit-bats and flower-bats.

There is in this case no question of chiropterophily. The ripe catkins are, however, eagerly sought after and consumed by *Cynopterus* species, as described by me (1935).

b) **Eriobotrya japonica**

This example probably rests on a similar error, as a fruit-eating bat is mentioned as pollinator.

c) **Cocos nucifera**

The old report of Moseley quoted by Porsch says that insect-eating bats were seen circling round the flowers. They undoubtedly prey upon the insects visiting the flowers, but this has nothing to do with pollination.

d) **Areca catechu**

This statement must also rest on some error of the kind mentioned under a) or c).

Many data reported in the list in which Pteropus is mentioned are doubtful, as the activity of this animal consists nearly always in the destruction of flowers and young fruits. In kapok-plantations it is for this reason regarded as a pest.

7. Musa

I present here the first photograph ever made of a *Macroglossus* in the act of visiting a flower (fig. 4). The animal is seen clinging to the still closed bracts of a male banana inflorescence and introducing its snout into an open flower, while forcing apart the perianth slips.

I have to thank Mr. J. Fersenaar (Bandung), who took this picture, for his cooperation.

The study of Cheesman (1947) shows that the classification of the species in the genus *Musa* according to the mode of pollination does not agree with the taxonomic groups in that paper. The section *Eumusa*, however, seems to be entirely chiropterophilous.

8. Flowerbats as a factor in plant communities

At an earlier occasion (1935, p. 17) I have already pointed out that flower visiting bats can only subsist in plant communities where all the year round chiropterophilous flowers are present. The reverse is just as true.

When we find in a region a chiropterophilous plant species with a limited flowering period, we may be certain that other chiropterophilous plants will be present with a different flowering period. The species are bound together by means of the bat—and in the case of the cave-dwelling bats by the presence of suitable caves.

Allen (1940) already drew the attention to this necessity of overlapping flowering periods. It might be of interest to test this in a community with a limited number of plant species, e.g. in the African savannah-regions where *Kigelia* and *Adansonia* are present as nuclei of bat-pollination. Probably some *Markhamia*, *Spathodea* and *Parkia* species will fill up the gaps. For Australian regions in which *Adansonia gregorii* occurs, the study of these relations might be an attractive task for a local biologist.

Porsch (1939) describes chiropterophilous *Cactaceae* (e.g. *Carnegiea* from Arizona) from regions with only some *Agave* species as possibly supplementary plants and with a winter period in which such flowers are entirely absent. If we cannot assume hibernation of the bats, we might, as the region borders on more tropical regions, think of migration (c.f. the humming birds).

It is of interest to note that, as Professor Chas T. Vorhies (Tucson) wrote to me, there is in Southern Arizona a species of nectar-feeding bat, viz. *Choeronycteris mexicana*. It probably migrates southward in the winter. According to Prof. E. Lendell Cockrum the same holds true for *Leptonycteris nivalis*.

I hope to describe elsewhere the role of fruit-eating bats in tropical

plant-communities, especially in those occurring near the sea. Their role is more conspicuous. In that study I shall include a distributional map.

9. Flowerbats and plant areas

We know some instances where the distribution of a plant species is limited by the distribution of its pollinators (and vice-versa).

One day we may be able to reconstruct the repopulation of Europe after the last glacial period by immigrants from warmer regions by taking into account the speed with which their pollinators and seed dispersers could follow them. In a forthcoming study on the leguminous pods I made a first attempt in this direction, and I pointed out a.o. that it is not the presence of cauliflory in the tropics, but its absence in Europe, that has to be explained, and that this explanation is to be sought in the direction just mentioned.

The interdependence is demonstrable only when there is but a single pollinator, and when we may be sure that there is no autogamy.

I pointed already (1936) to the case of *Kigelia* which in Hawaii is sterile because of the absence of bats. Why the *Adansonia digitata* cultivated there is fertile, I do not know. In this connection it is useful to consider the old question whether *Musa Fehi* is native to Hawaii. Apart from other considerations it seems clear that this chiropterophilous and chiropterochorous species cannot be native to an island in which there are no bats. An introduction from New Caledonia is for this reason a more probable assumption.

The eastern limit reached by many *Phanerogamae* that penetrated into the Pacific region should not be considered only from a historical and geological point of view, but also in relation with the distribution of flower bats and fruit bats, though this factor may not be independent.

A detailed study would necessitate the cooperation of some botanists and a zoologist. I will confine myself to some of the basal points.

The chiropterophilous plants are, so far as we know, limited to the region west of Fiji.

The Macroglossine bats—the nectar-feeders—are in Africa represented by the genus *Megaloglossus*. It is accompanied there by transitional forms included in the *Pteropinae*, like *Eidolon*, of which Jaeger (1954) has shown that it can act as pollinator.

In S.-Asia there are several genera, viz. a) *Macroglossus*, which reaches Indonesia, New-Guinea and the Bismarck Archipelago, b) *Eonycteris*, known from Tonkin, Siam, Birma, Malaya, Indonesia and the Philippines, c) *Melonycteris* and d) *Nesonycteris* which occur in the Salomons and the Bismarck Archipelago, e) *Notopteris* found up to New-Caledonia, the New Hebrides and Fiji, f) *Syconycteris*, a native of New Guinea, the Bismarck Archipelago and North Australia.

Small *Pteropinae* may take over their role, incidentally with regard to the main class of chiropterophilous plants, and obligatory with regard to the class which attracts the bats by means of solid food.

A study of the genus *Parkia*, which reaches in Fiji its eastern limit might give interesting results.

The chiropterophilous *Sonneratia acida* L.f. (*S. caseolaris* (L.) Engl.) does not go so far eastwards as most of the other mangrove plants, and remains within the area occupied by *Macroglossinae*.

The fertility of *Ceiba* on many eastern Pacific islands (Makatea, Niue, Rarotonga, Samoa, Marquesas) is undoubtedly due to the introduction of self-fertile forms, like those cultivated in Java.

The fertility of introduced *Ochroma lagopus* and *Crescentia cujete* in the Marquesas (F. B. H. Brown) and of *Durio zibethinus* and *Crescentia cujete* in Rarotonga (Wilder) deserves further study. The omnipresence of *Mucuna gigantea* in the Pacific islands shows that this species is a transitional form in chiropterophily, just as *M. pruriens*, which I already recognized as such (1941).

10. Behaviour of the bats and cauliflory

I already paid some attention (1936, p. 3) to the fact that *Macroglossinae* and *Pteropinae* are less dexterous in avoiding obstacles, which is due to their less efficient "radar"-system. The necessity of an exposed position of the chiropterophilous flowers therefore, is obvious.

Native hunters in Ambon (where bats are eaten) know that this kind of bat is more easily caught by means of nets and hooks than the other ones.

Land and Chapin (1917) already said (p. 483) "contrary to the behaviour of insectivorous bats the larger fruitbats, when let loose in a room, fly against obstacles". Tate (1942) reported from New-Guinea that the nectar-eating *Syconycteris australis* was the only species of bat, which was readily trapped in fowling nets at night. This study also describes the gradual change in skull structure and the reduction of teeth in resp. *Eonycteris*, *Macroglossus*, *Nesonycteris* and *Notopteris*.

The specimen figured in fig. 4 when coming back after the flash, took fright when it approached the flower on its second round, made a right turn, and collided with the camera tripod. Its repeated refusal to land afterwards on this inflorescence seems to prove the existence of a memory for the position of objects.

Notwithstanding these facts and the frequency of cauliflory and flagelliflory in chiropterophilous and chiropterochorous plants, some authors hesitate to accept the importance of cauliflory as an adaptation to bat visits. Of course, when we call a structual modification an adaptation, this does not mean that it is regarded as developed entirely de novo. Every change of this kind starts with materials, tendencies, and relations that are already present. On the other hand it seems to be an over-sensitivity to the teleologic point of view to regard an adaptation merely as an "*Ausnützung*", and that e.g. bats simply make use of morphologically conditioned, but in relation to them accidentally present, cases of cauliflory.

Porsch (1941) applies this reasoning to the cauliflory of *Eugenia cauliflora*, saying that it lies within the scope or variation of the genus. However, it seems to me that the cauliflory of many sister species may also be interpreted as ecologically conditioned by bonds between

themselves and bats. Such bonds doubtless exist, for these plants are chiropterochorous.

When we see how in myrmecochorous plants the whole organization may be changed in order to bring the ripe fruits and seeds nearer to the ground and within the grasp of the ants, it is strange that the changes in position of bat flowers resulting in an easier accessibility to these animals are not generally recognised as adaptations.

In this connection it has been argued that the fact that cauliflory is limited to the tropics, does not rest on an ecological cause, but that it merely is a remnant of a primitive morphological structure. This may be true in some cases, but cauliflorous chiropterophilous plants like *Kigelia*, *Crescentia*, *Parmentiera*, *Eugenia*, *Amphitecna*, *Durio*, *Mucuna* and many *Sapotaceae* seem to be far from primitive.

The investigations of Mc Lean Thompson (1946, 1951) with regard to the anatomical background in various cases of cauliflory have shown that the character can not be regarded as primitive and that there is no homology, but that to the contrary cauliflory is a secondary character; it is an example of a convergence obtained by the most divergent means.

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NOTES ON STELLARIA NEMORUM L.

STELLARIA NEMORUM L. SUBSP. GLOCHIDISPERMA MURBECK IN THE NETHERLANDS

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INTRODUCTION

Stellaria nemorum L. is a plant of rare and local occurrence in the Netherlands, growing in woods.

With a few exceptions (Leiden, Dordrecht 1915) it is restricted to the eastern part of the country, mainly to the very southeast (southern district of the province of Limburg) and the central northeast (near the village of Norg in the province of Drente).

When collecting specimens in Limburg and Drente in 1950 I found them to be of mutually different appearance, especially on account of the indumentum of stems and leaves and of the length of the leafstalks. It was judged worth while to continue and to extend the study of the two populations.

The late professor Siertsema had already noticed the existence not of one but of two taxa in the Netherlands and on the labels in the Leiden herbarium had named them *Stellaria nemorum* L. and *Stellaria nemorum* L. subsp. *glochidosperma* var. *laevipes*, — (subdivisions without names of authors) —, the latter of which was new to the Netherlands. As far as we know, however, he did not publish his findings.

A closer and comparative study of european herbarium and of the literature led us to nearly similar results and made it clear that representatives of two types of *Stellaria nemorum* do occur in the Netherlands. The two taxa might even be conceived as two separate, yet closely related species, which would depend on the investigator's opinion on species limits. We found already many differences but, since results of breeding experiments are not available yet in sufficient quantity, we would not make any statement on taxonomic rank of the two groups here. We leave them in the rank of subspecies, as it was generally done by workers on the present subject.

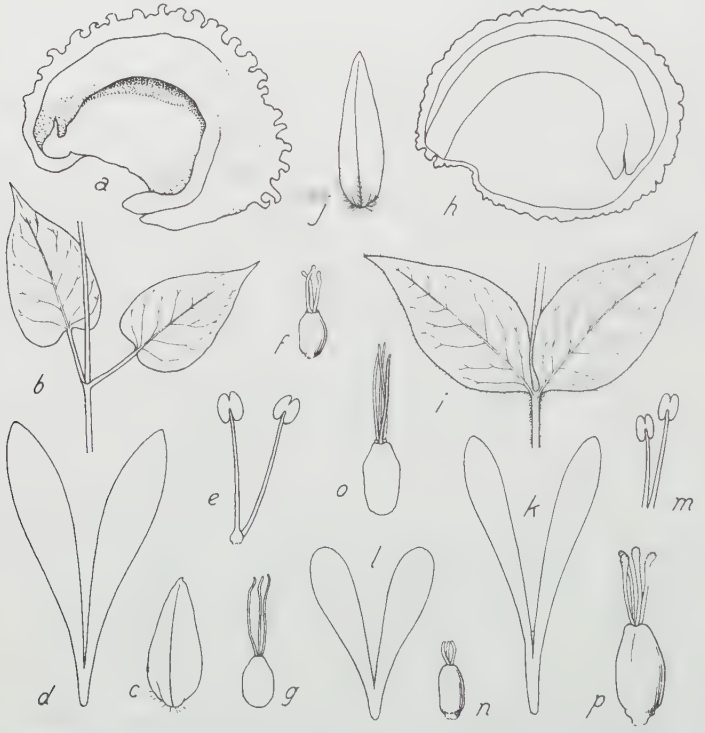
However, publication of the data obtained so far on *Stellaria nemorum* in the Netherlands might be desirable at present, as it may mean a connecting link between recent papers on the same subject from Great Britain and Belgium. Our data concerning the Netherlands fill up a gap in the known area of the subspecies *glochidisperma* Murb., having been extended recently in France, into Spain, LAWALRÉE (1953 *a*, *b*) and into Great Britain, GREEN (1954).

For the sake of a comprehensive terminology it is desirable to state in advance that the Limburg specimens are considered to belong to *S. nemorum* L. subsp. *nemorum*, whilst the plants of Drente, few doubtful specimens excepted, belong to *S. nemorum* L. subsp. *glochidisperma* Murb.

During the present studies we made use of the herbaria of Groningen, Leiden (State herbarium and herb. of the Roy. Bot. Soc. of the Netherl.), Utrecht, Wageningen. Our thanks are due to dr V. Westhoff and mr H. Doing Kraft for valuable ecological information.

Description and discussion

Although Murbeck was not the first to distinguish various types of *S. nemorum* L., he was the first to publish a detailed comparative description of the two taxa, which in his opinion deserved the rank of subspecies. He summed up quite a number of differences between his subspecies *glochidisperma* and the type-comprising taxon concerning colour of plant, height of stems, number of stolons, indumentum, presence or absence and length of petioles, shape of leaves, size



Stellaria nemorum L. a-g, subsp. *glochidisperma* Murb.: a, seed (section, 33 1/3 ×); b, uppermost pair of leaves under inflorescence (2/3 ×); c, calyx leaf; d, petal of early flower; e, stamens; f, g, pistils in various stages of development; (c-g 3 1/3 ×). h-p, subsp. *nemorum*: h, seed (section, 33 1/3 ×); i, uppermost pair of leaves under inflorescence (2/3 ×); j, calyx leaf; k, l, petals of early and later flower resp.; m, stamens; n, o, p, pistils in various stages of development; (j-p 3 1/3 ×).

of bracts, position of main pedicels at fruiting stage, size of fruit in relation to calyx, appearance of seeds.

Most of the differences mentioned are also evident on comparison of the two main dutch populations. The bending down of the main fruit stalks, however, is not restricted to subsp. *nemorum*, but also happens to take place in subsp. *glochidisperma*. On the other hand we discovered some more differences, especially in flower characters, in the dutch material at least. [1] All these differences, being quanti-

Characters of *Stellaria nemorum* L.

subsp. *nemorum*	subsp. *glochidisperma* Murb.
plants higher, paler green; tend to be red when young, only in basal parts of basal internodes	plants lower, darker green; tend to be rather red when young, especially in lower internodes
stolons not so numerous	stolons numerous
stems usually thicker	stems usually thinner
leaves solid, longer and narrower (relatively at least), slightly cordate to cuneate at base, stalked (lower leaves) to sessile (upper leaves); leaves at node under first branching of inflorescence usually sessile	leaves delicate, shorter and (relatively) wider, obviously cordate at base, often with undulated margin, stalked to sessile, stalks relative to leafblades longer; leaves at node under first branching of inflorescence usually stalked
indumentum ± rich, also on basal parts of plant	indumentum less developed, basal parts of plants almost glabrous, except when young
bracts gradually diminishing in size	bracts abruptly diminishing in size
calyx bowl- to funnel-shaped	calyx bowl-shaped
sepals more narrowly elliptical to ovate, bearing bristles and glandular hairs	sepals somewhat bigger in size, identical in shape or relatively wider, less pubescent, particularly in matters of glands
corolla smaller [2]	corolla larger
petal lobes spatulated, narrow; widest zone near to the top	petal lobes spatulated, wider; widest zone not so near to the top
filaments falcate, slender; glands at base of episepalous filaments not so obvious	filaments falcate, but not so slender; glands at base of episepalous filaments obvious
anthers smaller	anthers larger
ovary cask-shaped	ovary egg-shaped
styles (and stigmas) slender [3]	styles (and stigmas) not so slender [3]
fruits at most twice as long as calyx	fruits usually twice as long as calyx
edge of ripe seeds furnished with hemispherical to cylindrical and unarmed tubercles	edge of ripe seeds furnished with long, cylindrical or conical papillae with barbate caps
flowering time earlier, (May and June in the Netherl.)	flowering time later (June until the middle of July in the Netherl.)
habitat: moist forest on rich soils, pH ± 6-7	habitat: dryer forest on poorer soils, pH ± 4-5
chromosomes 2n = 26 (counted by Peterson)	chromosomes 2n = 26 (Peterson)

[1] On my request preliminary studies were made by miss C. S. Duintjer.
[2] to be considered at time when both subspecies are flowering.
[3] Comparison with length of ovary is difficult, as their mutual length ratio depends on stage of development.

tative in nature, are obvious on comparison of the two taxa, but the characters are probably not so easy to handle in a study of one single subspecies.

The known differences, as presented by several workers and as they were met with in the dutch plants, may be listed in the foregoing table.

A brief discussion is needed concerning several paragraphs of the present list.

In my opinion the index and shape of the leaves in *S. nemorum* are on the whole usable as diagnostic characters in comparable stages of full-grown plants. Yet difficulties remain and we should point here to remarks by P. S. GREEN in his paper (1954), who established a great deal of overlapping. Thus a discrepancy between the usefulness of the character in fieldbotanical research and the results of statistical calculations may exist. In any case is there sense only in comparing leaves at corresponding nodes, the node under the first branching of the cyme being preferable for that purpose. Moreover the leaves at that node are usually sessile in subsp. *nemorum*, while stalked in subsp. *glochidispermà*.

The decrease in size of bracts — gradual in subsp. *nemorum*, abrupt in subsp. *glochidisperma* — is a good diagnostic character and the same holds for the relief of the edge of the seeds, mentioned above (see also GREEN, 1954).

HEGI (1911) denied the occurrence of papillae with barbate caps in subsp. *glochidisperma* Murb. It was one of his motives, — an invalid one — to the rejection of the subspecific epithet, which he thought inappropriate, in favour of *circaeoides* Schwarz. The other reason was a question of supposed priority, to be dealt with below.

The difference in size of flowers observed probably is mainly due to difference in flowering time, combined with the phenomenon of the earliest flowers being larger than those developed towards the end of the season. Thus, by the time when subsp. *glochidisperma* starts to unfold its first flowers, which have maximal size, subsp. *nemorum* already bears its later and smaller flowers.

Difference in flowering time between the two taxa was already established by PIERRAT in France, who recorded a difference of ten days at least, his *S. montana* (= subsp. *glochidisperma* Murb.) being the latter to unfold its flowers. Still greater difference is sometimes evident in the Netherlands, where it may amount to about 3 weeks. Culture experiments in the University botanic garden "de Wolf" have revealed that this is not a mere question of latitude; even after several years of cultivation in one locality the difference in flowering time continues to exist. Thus there seems to be rather an effective barrier to gene exchange, a restriction of possibilities for such an exchange at least, even in localities where the two taxa might grow side by side in nature.

The principal area of *S. nemorum* subsp. *nemorum* in the Netherlands lies in the very south-east of the country, S.-Limburg. However, few specimens of various other localities are present in the Leiden herbarium, coming from more central-eastern, central and central-

western districts (Roermond-Beegden, Denekamp (coll. 1911), Heerwaarden, Dordrecht (1915), Leiden). Subsp. *glochidisperma* is known so far from Norg only.

When considering the two main areas of distribution of the two subspecies in the Netherlands, one might expect geographical causes of distribution. There is a difference in latitude, the distance between Norg and southern Limburg being almost 200 miles in a north-south direction. Without further study we might take the difference in latitude also as a ready explanation of difference in flowering time; such a difference of ca 2 weeks is evident in various phenomena with many species of the dutch flora. We have learnt, however, from our cultivation experiments that such an explanation does not hold for *Stellaria nemorum*; its flowering time appeared to be genetically fixed.

Moreover, the position of the other localities, mentioned above, and a comparison of the whole european area of the two subspecies make us reject a geographical explanation. Both taxa are now known from the greater part of Europe; the borderline of the area of distribution of the species as a whole runs through Sweden, Norway, Great Britain (Wales), France, Spain, Corsica, Italy, Yugoslavia and probably Russia. It should be remarked, however, that extensive information on the two subspecies separately is not available. In central Europe the species is mainly recorded from montane and subalpine regions; only few alpine localities are known (800 ft., subsp. *nemorum* 860 ft.). It also occurs in lower regions. More chorological details are desirable.

Concerning the known area of subsp. *glochidisperma*, Spain Lawalrée, 1953 *b*), Great Britain (Green, 1954) and the Netherlands (Siertsema in sched. 1935, Andreas, 1955) could be incorporated only recently. In Scandinavia subsp. *nemorum* goes further north than subsp. *glochidisperma* (Hultén, 1950). Thus, there is no important divergence in geography of the two subspecies; they are considerably sympatric from that point of view.

Nevertheless barriers of some kind seem to be active in generally keeping the two taxa separate; putative hybrids are rare as known so far. Hegi mentioned „Zwischenformen" (1911*a*), Green considered a few specimens in british herbaria to be such hybrids (1954), while Peterson (1936) stated to have obtained hybrids artificially; in the Leiden herbarium few doubtful specimens from Norg are present. But, keeping in mind the nature of the characters listed above, we may understand that such hybrids are by no means easy to be distinguished.

After having treated in brief morphological data, the partial effectiveness of genetically fixed biological barriers and the area of distribution of the species, we now turn to ecological barriers, which possibly are important in determining the details of the distribution pattern of *Stellaria nemorum* and in keeping both subspecies separate.

Ecology

Ecological (including phytosociological) conditions are evidently dissimilar in the two main dutch localities. Some years ago dr V. Westhoff in a letter kindly communicated details. Revision and enlargement of his then description in collaboration with mr H. Doing Kraft brought his notes into line with recent views and resulted in the following ecological paragraph.[1] I am indebted to both and especially to dr V. Westhoff for his permission to include it in my paper.

He described the forest of Norg as a plant community near to the north eastern dutch geographical variant of Querceto-Betuletum s.s. The association of Querceto-Betuletum s.s. indicates a poor sand soil or loam with a low base status, a low biological activity, a low pH ($\pm$ 3–4), raw humus and a gray-brown podzolic profile. The geographical variant mentioned above, marked a.o. by abundance of *Ilex aquifolium* and *Corydalis claviculata* (atlantic features!) and by the occurrence of *Luzula pilosa*, is a vegetation type indicating an elevated humidity of the air, which, in its turn, may be an atlantic as well as a montane character. Indeed, this variant presents a transitional form between the pure, poor Querceto-Betuletum of lowland sands (the former Querceto roboris-Betuletum) and the submontaneous Quercetum sessiliflorae (previously named Querceto sessiliflorae-Betuletum). The circumscription of the forest of Norg as a plant community "near to" this north-eastern dutch variant is due to the circumstance, that the abundance of *Oxalis acetosella*, *Corylus avellana* and *Milium effusum* and the occurrence of *Stellaria holostea* and *Anemone nemorosa* in the forest of Norg indicate a tendency to the woodland associations of the Querceto-Fagetea, i.e. they indicate a habitat with a richer soil than it is found within typical Querceto-Betuletum: finer texture, higher base status, higher biological activity and a tendency to crumb structure. The whole qualitative and quantitative floristic assemblage of this transitional form further indicates a pH of the soil (in a depth of 20 cm) of 4–5, a moist soil with an A-G-profile and somewhat raw humus.

To provide more detailed ecological information dr Westhoff procured the following ecological sample plot analysis taken by him after the method of Braun-Blanquet, i.e. a species list arranged to vegetation structure, and presenting quantitative data about the abundance and dominance of the individuals. A description of the soil profile is added.[2]

Number of the sample plot analysis: V.W. 41–128. Date: July 1941. Locality: forest of Norg, part W. of the way Norg-Huis ter Heide. Aspect: heavy oak wood.

[1] Without responsibilities on our side, except for some minor details — Ch.H.A.
[2] My own plant list, now being superfluous, has been deleted. — Ch.H.A.

Surface studied: 100 m²

High tree layer: 90 %, 15 m high.			
Quercus robur L. (4 ex.)	5		
Low tree layer: 30 %, 6–7 m high.			
Ilex aquifolium L.	3.2		
Shrub layer: 40 %, 2–4 m high.			
Sorbus aucuparia L.	2.2	Ilex aquifolium L.	2.1
Corylus avellana L.	2.1	Frangula alnus Mill.	+.1
Herb layer: 80 %.			
Rubus fruticosus L. coll.	4.2	Ilex aquifolium L.	+.1
Oxalis acetosella L.	3.3	Sorbus aucuparia L.	+.1
Convallaria majalis L.	2.1	Luzula pilosa Willd.	+.1
Holcus mollis L.	2.2	Pteridium aquilinum Kuhn	+.1
Maianthemum bifolium F. W. Schmidt	1.2	Corydalis claviculata Lam. et DC.	+.2
Milium effusum L.	1.2	Hedera helix L.	+.2
Stellaria nemorum L.	+.2	(Melandrium diurnum Fr.)	
Stellaria holostea L.	+.2	Anemone nemorosa L.: died away.	
Moss layer: 5 %.			
Dicranum scoparium (L.) Hedw.	+.2	Polytrichum attenuatum Menz.	+.2

Outside the studied plot in other parts of the forest the following species occurred moreover:

Picea abies Karsten (planted), Fagus sylvatica L., Betula pubescens Ehrh., Lonicera periclymenum L., Ajuga reptans L.

Soil profile: A_0: 2 cm fresh litter; A_1: 4 cm decaying litter; A_2: 15 cm black humus; G: gray-black sand with white grains, gradually getting more pale to the depth.

In southern Limburg dr Westhoff never saw *S. nemorum* in a like vegetation; there the species (subsp. *nemorum*) is exclusively met with in the Pruneto-Fraxinetum on alluvium along brooks. This association belongs to the alliance of Alno-Ulmion (class of Querceto-Fagetea) and is for the greater part synonymous with the subassociation Querceto-Carpinetum filipenduletosum, an older and better known name. Those habitats are more fertile than the other ones assigned to the class of Querceto-Fagetea, and they are extremely fertile compared with the habitat of the forest of Norg. They have mild humus and a rather wet soil being rich in carbonates and presenting a high base status and a well-developed crumb structure, their pH amounting to 6–7. Dr Westhoff communicated the following ecological sample plot analysis (see above):

Number of the sample plot analysis: V.W. 42–49. Date: August 2, 1942. Locality: Forest S. of Slenaken at the left edge of the small river Gulp, Southern Limburg, quite near the Belgian frontier. Soil: black wet humus more than 20 cm deep. Phreatic level 10–40 cm below surface.

Aspect: Poplar forest.
Surface studied: 200 m²
High tree layer: 70 %, 20 m high.
Populus L. spec. culta 1
Low tree layer: 30 %, 10 m high.
Alnus glutinosa Gaertn. 3
Shrub layer: 60 %, 2–3 m high.

Alnus glutinosa Gaerth.	4.2	Rosa canina L.	+.1
Fraxinus excelsior L.	2.1	Prunus avium L.	+.1
Corylus avellana L.	2.1	Ribes uva-crispa L.	
Euonymus europaeus L.	+.1	Crataegus oxyacantha L.	+.1

Herb layer: 100 %, up to 2 m high.

Rubus fruticosus L. coll.	2.2	Polygonatum multiflorum All.	+.2
Stellaria nemorum L.	2.3	Epipactis helleborine Crantz	+.1
Urtica dioica L.	2.3	Humulus lupulus L.	+.2
Aegopodium podagraria L.	1.1	Crataegus monogyna Jacq.	+.1
Impatiens noli-tangere L.	1.3	Agropyrum caninum P.B.	+.2
Festuca gigantea Vill.	1.2		
Filipendula ulmaria Maxim.	1.1	Geum urbanum L.	+.2
Galium aparine L.	1.1	Ficaria verna Huds.	+.2
Melandrium diurnum Fr.	1.1	Primula elatior Grufb.	+.2
Lamium galeobdolon Crantz	1.3	Chrysosplenium oppositifolium L.	+.3
Brachypodium silvaticum P.B.	1.3		
Ribes silvestre M. et K.	1.2	Hedera helix L.	+.1
Phalaris arundinacea L.	1.2	Valeriana officinalis L.	+.1
Quercus robur L.	+.1	Equisetum arvense L.	+.2
Corylus avellana L.	+.1	Galeopsis tetrahit L.	+.1
Fraxinus excelsior L.	+.1	Solanum dulcamara L.	+.1
Dactylis glomerata L.	+.2	Dryopteris austriaca (Jacq.) Woynar	+.1
Rumex obtusifolius L.	+.1		
Stellaria holostea L.	+.2	Angelica silvestris L.	+.1
Geranium robertianum L.	+.2	Vicia sepium L.	+.1
Heracleum sphondylium L.	+.1	Polygonum bistorta L.	+.2
Stachys silvatica L.	+.2	Rubus idaeus L.	+.2

Moss layer: 10 %.

Eurhynchium Br. eur. sp.	2.3	Atrichum undulatum (L.) P.B.	+.2
Fissidens taxifolius (L.) Hedw.	+.2	Lophocolea bidentata (L.) Dum.	+.2

To this information dr Westhoff adds some notes on his experience abroad about *Stellaria nemorum*. On the 9th of October, 1955, he collected *Stellaria nemorum* ssp. *glochidisperma* in a wet mountain alder woodland of *Alnus glutinosa* (Alnetum glutinosae cardaminetosum) at 750 m altitude, near the small town Altenau in the Picea-zone of the central european mountain of Harz, situated at the N.E. limits of Western Germany. *Stellaria nemorum* was thriving there abundantly; main companion species were *Carex elongata* L. (dominant), *Carex remota* L., *Stellaria alsine* Grimm., *Deschampsia caespitosa* P.B., *Chrysosplenium oppositifolium* L., *Chrysosplenium alternifolium* L., *Phalaris arundinacea* L.

Edaphically this habitat is intermediate between that of *S. nemorum* subsp. *nemorum* (southern Limburg) and that of subsp. *glochidisperma* (Drente) in the Netherlands; it is much wetter than the latter (as waterlogged as the former), but it is poorer in nutrients than the former and equals the latter in that respect. Moreover, it corresponds with the latter in climatic respect: more elevated precipitation, high air humidity and cool summers (common features of montaneous and atlantic climates).[1]

The conclusion that each of the two subspecies has its own ecological preference was arrived at not only by an analysis of the natural habitats, but also by the results of cultivation experiments. In the botanic garden "de Wolf" a great variety of environments has been created and is kept up, so that many plant species may find suitable conditions for their development and the transplant of particular plants tends to be successful. We began planting specimens of the two subspecies in one habitat, a deciduous wood along a brooklet on rather moist and rich soil, pH = ± 6.1. Subsp. *nemorum* thrived well and spreaded, whereas subsp. *glochidisperma* had disappeared after about three years, so that fresh material had to be introduced. These experiments are to be continued.

Ecological indications on herbarium labels, if present, usually are too brief to procure knowledge of habitat conditions. The few comprehensive notes, however, dealing with subsp. *nemorum*, agree in the point of moist habitats.

Although Hegi (1911 *b*) presents rather detailed information on ecological conditions of central-european habitats, such data are usually scanty. *S. nemorum* is frequently but not exclusively met with in deciduous woods, where it gives preference to the presence of *Alnus* species. Subsp. *glochidisperma* in northern Europe preferably grows in beech forests (Murbeck 1899, Hegi 1911*b*); Schwarz (1897) considers it a diverging form, originating in shady localities.

We need detailed ecological particulars on the various habitats, especially from localities where the two subspecies occur not too far apart, which is probably the case in Luxemburg. Moreover, we should look out for hybrids, mentioned already by Hegi (1911*a*) and Green (1954). More detailed information in these fields might help us to gain a better insight also in the micro-evolutionary development of the units and their present taxonomic status, not to be discussed here.

Nomenclature

Stellaria nemorum was named and described by Linnaeus (1753). In 1880 the french naturalist D. Pierrat described a new species, closely related to the foregoing *S. nemorum*, under the name *S. montana* Pierrat sp. nov.

S. Murbeck (1891) was the first to publish *S. glochidisperma* as a subspecies under *S. nemorum* L. Although under the present Code of Nomenclature (art. 34) the binary combination of the subspecific

[1] Here ends dr Westhoff's communication.

epithet with the genus name is not admissible, it is reasonable to accept it here, while Murbeck clearly indicated that a subspecies of *S. nemorum* was meant. Freyn (1892) apparently gave species rank to the taxon under the name *Stellaria glochidisperma* Murb. In a subsequent paper (1899) and again in a binary combination Murbeck named his subspecies *S. glochidosperma*, thus changing *i* into *o*. yet without any explanation. We cannot accept, however, the latter orthography of the subspecific epithet for the following reasons.

From Murbeck's description it is evident that his epithet referred to the barbed tubercles of the seedcoat of the plant under consideration and thus should have been based on the greek word glōchin or glōchis. Consequently *glochinosperma* would have been the correct spelling, as Hegi (1911a, b) already stated. We are not entitled, however, to alter a name for etymological reasons, whilst the original spelling must be retained except in case of typographical or orthographic errors (art. 82, Code). Thus, in my opinion, *glochidisperma* Murb., which is the earliest subspecific epithet in its original spelling, should be retained; it should not be replaced by *glochidosperma* Murb., which might be considered to be either orthographic aberrant or a later synonym.

Hylander (1945), however, seems to hold a different opinion; he uses the epithet *glochidosperma* Murb., as did many authors.

Hegi (1911*a*), — (see also p. 148 of the present paper) —, when defending Schwarz's epithet *circaeoides* of 1897 by reasons of priority, referred to Murbeck's paper of 1899 only, but he apparently overlooked Murbeck 1891. Moreover this epithet is of uncertain taxonomic rank, as its author applied it to "eine habituell sehr abweichende Form: β circaeoides A. Schwarz. And his information "ad amicos" of 1881 means an ineffective publication.

Murbeck (1891) does not make any mention of Pierrat's *S. montana*. Yet, in 1899 he divided *S. nemorum* L. into 2 subspecies, viz. *S. glochidosperma* Murb. and *S. montana* Pierrat. (According to the present rules the latter should have been signed (Pierrat) Murb., because of change of taxonomic rank).

From 1899 on and up to the present time these two names were conceived as indicating two mutually different subspecies, *glochidisperma* (or *glochidosperma*) divergent from the Linnean type, *montana* comprising it. This interpretation, however, was incorrect.

Pierrat's original description, which appeared in C. r. de la Soc. bot. Rochelaise II (1879), p. 58 (La Rochelle — 1880), under the title "Note sur le Stellaria montana Pierrat sp. nov." may be fully quoted here.[1]

"Cette plante diffère du Stellaria nemorum L. par une taille moins

[1] On mr P. Jovet's request mr L. Rallet kindly procured on my behalf a handwritten copy of the original description by Pierrat and some details on its author. Pierrat was a french naturalist, who lived at Gerbamont, Vosges. From 1878 to 1892 he was a member of the "Soc. bot. Rochelaise", which was a botanical exchange club.

élevée, une floraison plus tardive, au moins dix jours, à la même altitude, et surtout par les caractères suivants:

Feuilles moins larges et moins profondément en cœur à la base, pétioles moins bordés, pétales à division plus longues, plus étroites et plus acuminées au sommet; capsules longues.

Elle n'affectionne pas les lieux humides et les cours d'eau comme Stellaria nemorum".

This copy of the description is exactly identical with the printed diagnosis on the herbarium sheet with *S. montana* of Kew, signed by Pierrat and published in photograph in a recent paper by P. S. Green '1954'. It should be remarked that the name *Stellaria montanum* (not montana) figures on the printed label of Soc. Roch., added to that sheet.

Although there are some discrepancies (leaf shape) in the diagnosis, which is somewhat inadequate, it seems evident that *S. montana* denoted the same taxon as the one which Murbeck was to name subsp. *S. glochidisperma* ten years later. Thus, *S. montana* is not a synonym of *S. nemorum* L. subsp. *nemorum*, but of *S. nemorum* L. subsp. *glochidisperma* Murb., (see also Green 1954).

There is, however, no need for nomenclatural change as long as we consider the two taxa to be subspecies, for the epithet *glochidisperma* Murb. was the earliest having been published in subspecific rank.

As in this paper we do not intend to make any decision on taxonomic rank yet, we shall follow previous writers here in retaining two subspecies, the names of which should be *Stellaria nemorum* L. subsp. *nemorum* and *Stellaria nemorum* L. subsp. *glochidisperma* Murb .(= *S. montana* Pierrat). Both subspecies now are known from the Netherlands.

SUMMARY

A study on *Stellaria nemorum* L. in the Netherlands was made concerning morphology, geography, ecology and nomenclature. The species has usually been divided into 2 subspecies, the nomenclature of which is dealt with. The correct names appear to be *S. nemorum* L. subsp. *nemorum* and *S. nemorum* L. subsp. *glochidisperma* Murb. The known area of distribution of subsp. *glochidisperma* Murb. has been extended into the Netherlands; representative populations of both taxa were found. The two subspecies are mutually different not only in many morphological properties, but also in flowering time and probably in ecological preference. Cultures and breeding experiments were made and are still in progress.

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SOME FACTORS INFLUENCING THE ABSCISSION OF DEBLADED LEAF PETIOLES

BY

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Introduction

This reasearch was prompted in the first place by some questions which had arisen in the practice of fruit growing. It is generally known that there is some relation between the growth of a tree and its fruitfulness, the highest fruitfulness being obtained at a moderate growth of the tree (see e.g. Kobel, 1954). Now one of the factors determining fruitfulness is the fall of the young fruits. This fall is said to be stimulated by vigorous growth of the tree.

It is a common practice among fruit growers to bend the branches of too fast growing trees in a horizontal position, which is said to cause a reduced growth and an increased and earlier fruitfulness of the tree. According to some fruit growers this bending not only stimulates flower formation but also reduces the fall of young fruits. However, there is no agreement on the latter point.

It seemed desirable to investigate whether this common orchard practice could have some influence on abscission phenomena. Now experimenting with young fruits, for instance apples, is hardly practicable, as they are available only for a short time of the year and cannot easily been grown in large numbers under controlled conditions. It seemed acceptable, however, that the fall of young apple fruits is controlled by a similar mechanism as governs the fall of leaves. Some arguments for this supposition are the following:

a. The anatomical differentiations, accompanying the fall of apples, namely dissolution of the middle lamella between two layers of cells, either or not preceded by cell divisions (Mc Cown, 1943) do not differ from those observed in leaves (Addicot and Lynch, 1955).

b. Like debladed petioles, pedicels of apples fall after a certain number of days if the young fruit is removed, and falling can be inhibited by the application of growth substances (Barlow, 1948, 1950).

c. A healthy leaf is supposed to remain attached to the plant as a result of its continuous auxin production. This may also be true for apple fruits: it could be shown (unpublished results) that in young

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apple fruits a considerable auxin production begins immediately after fruit setting, whereas Luckwill (1948, 1953) provided evidence that periods of fall coincide with a low auxin content of the seeds.

d. According to Edgerton (1947) fruits of apples give the same general abscission reactions as do the petioles to various synthetic growth substances.

It seemed justified, therefore, to investigate the problems mentioned, for the present, with leaves. As an experimental plant *Coleus* was chosen, as this plant can easily be cultivated and has already been used in many investigations concerning abscission phenomena. Some of the results have been checked using leaves of apple trees and seedlings.

Material and methods

For the experiments a clonal stock of *Coleus rhenaltianus* was used. The plants were cultivated in a greenhouse at a constant temperature of 26° C, the experiments were carried out in a greenhouse at a constant temperature of 23° C. In general the deviation from these mean temperatures did not exceed 1° C. In the winter months (beginning November 1 st) the daylight was supplemented with the light of 60 Watt incandescent lamps (about one lamp per m^2 at a height of 75 cm) until the total length of the light period was $15\frac{1}{2}$ hours. In this way flower formation could largely be prevented. Although no fundamental differences were found between the experiments made in summer and winter, there were indications for quantitative differences (see experimental results).

In general, plants were used which possessed four or five full-grown leaf pairs (i.e. leaf pairs in which the leaf blades had reached or nearly reached their full size). If not stated otherwise, the youngest full-grown leaf pair (called number 1) with its axillary buds was wholly removed, the fall of the petioles of the second and third leaf pair (numbers 2 and 3) was studied. There were usually one or two pairs of unfolded leaves above the first fullgrown leaf pair. Together with the apical bud they will be called the tip of the plant. The length of the experimental petioles was about 1 cm, their axillary buds were removed. The tendency of the petioles to abscise was checked daily by lightly touching them from below with a small stick.

In the experiments with plants placed in a horizontal position a hook was put just behind leaf pair 1 in such a way that this leaf pair lay parallel to the soil surface, while the internode between the second and third leaf pair was supported by a small piece of wood in order to prevent this part from bending down. The axis of the second and the third leaf pair were perpendicular and parallel to the soil surface respectively. After one day the tips of the plants so treated had curved upwards and the operations needed for the experiment could be performed.

In the experiments with indole acetic acid (IAA) this substance was applied to the plant as a lanolin paste. The pastes were made by adding equal parts of water-free lanolin and an aquous IAA-

solution and mixing them by vigorous stirring in a waterbath at 45° C and afterwards at room temperature until cooled. In the controls a lanolin-water mixture, prepared in the same way, was used. The pastes were stored in the refrigerator and were never used for more than two weeks after their preparation. In all experiments the fresh cut surfaces of the petioles were covered with a small amount of the lanolin-water emulsion.

The control experiments with apples were made outdoors with shoots of "James Grieve" budded upon the rootstock E.M.IX, or with six months old seedlings from "Keulemans", cultivated in the greenhouse. For the experiments a number of petioles in the middle of the shoots were used.

The experimental results have been presented in time-course graphs, from which the number of petioles used can be read. In order to avoid long descriptions of each experiment the treatment of the plants has been drawn schematically in each graph. Only the full-grown leaf pairs have been drawn separately, the young leaves and the apical bud being taken together. A point marks the place where a full-grown leaf with its axillary bud has been wholly removed. A short line in the axil of the leaves denotes an axillary bud.

Results and conclusions

A. Experiments with plants in a normal position

a. Influence of the tip and axillary buds on the abscission of petioles

Four sets of plants were treated in the following way:

1. Removal of axillary buds in leaf pairs 2 and 3.
2. Removal of the tip.
3. Removal of axillary buds in leaf pairs 2 and 3, and the tip.
4. Control (untreated plants).

In all sets the leaf blades of leaf pairs 2 and 3 were removed and the fall of their petioles checked. The results are presented in Fig.1. The following conclusions can be drawn: the growing tip has a marked fall-accelerating action upon the petioles below (as has also been found by Jacobs (1955). The influence of axillary buds is negligible unless the tip has been removed. In the latter case the axillary buds probably take over the function of the tip. In order to prevent complications, in all subsequent experiments the axillary buds of leaf pairs 2 and 3 were removed.

The statement of Jacobs (1955) that IAA can fully replace the growing tip could be confirmed. The concentration used in our experiments was 2.10^{-3} g/cm^3, the pastes were renewed every other day. As this concentration was considered to be fairly high a lower concentration was tried, namely 2.10^{-6} g/cm^3, but in this case no effect could be obtained.

The influence of the growing tip has been checked in *one* experiment with apple leaves. Of twenty growing shoots of 8 years old apple trees ("James Grieve") four leaf blades in the middle of the shoot were removed. In one half of the shoots the tip was taken away.

Fig. 2 shows the result: just as in Coleus the growing tip in apples seems to accelerate the fall of the petioles below.

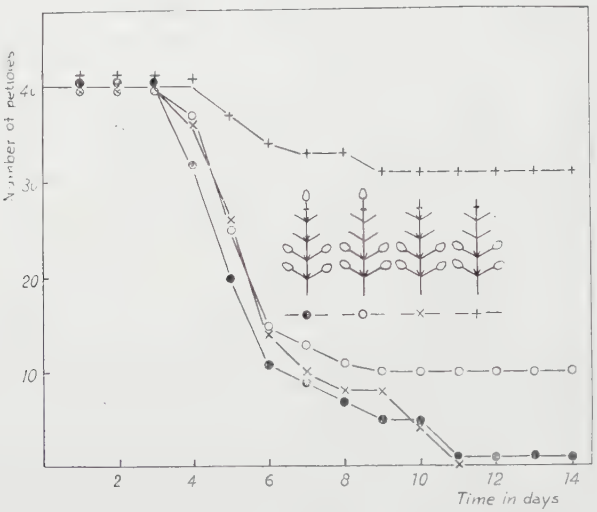

Fig. 1. The influence of the tip and axillary buds on the abscission of petioles in Coleus.

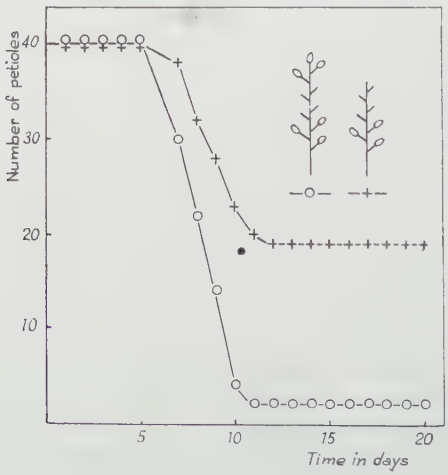

Fig. 2. The influence of the tip on the abscission of petioles in apple.

b. Influence of leaves and roots on the abscission of petioles

Rosetter and Jacobs (1953) and Jacobs (1955) found that, in addition to the tip, full-grown leaves above and below the debladed leaves can accelerate the fall of their petioles. This could be confirmed. Jacobs (1955) stated, that the fall-accelerating influence of leaves

below the petioles tested is almost entirely due to the stimulating effect they have on the compensatory growth of the young leaves in the tip ("A-leaves"). However, JACOBS did not test the influence of the lower leaves in the absence of the apical bud. In the following experiments the influence of the lower leaves (together with their axillary branches) was studied both in the presence and absence of the tip. In order to increase the effect of the remaining parts, the plants were left for two days after cutting off leaves and tips with the leaf blades still on leaf pairs 2 and 3. After this interval these leaf blades were removed and the fall of their petioles was checked. The results are shown in Fig. 3. It can be seen from this figure that,

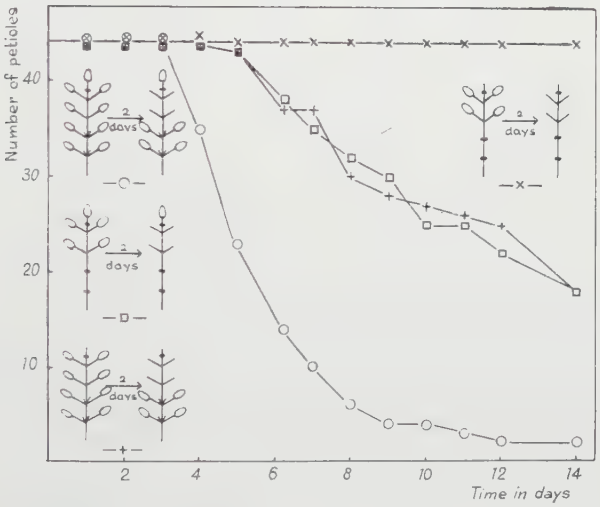

Fig. 3. The fall-accelerating action of the lower leaves on petioles in plants with and without tips.

also in the absence of the tip, there is a marked fall-accelerating influence of the lower leaves together with their axillary branches on the petioles tested. (The influence of the leaves alone have not yet been investigated). It was a somewhat striking result that the petioles of the wholly debladed plants did not or nearly not abscise. Since the publication of ADDICOT *et al.* (1949) leaf abscission is often studied by working with explants, in which case no leaves but only the petioles to be tested are present. In these experiments a rapid fall of the petioles was observed. It was supposed that the presence of the roots in our experiments might have some influence. Therefore, in a set of plants, both leaves and roots were cut off, the remaining stalks were put with their lower ends in vials containing about 50 cm^3 tap water. The water was renewed every other day. The plants remained quite healthy in appearance. Again there were two days between the deblading and derooting of the plants and the deblading

of the leaf pairs 2 and 3 to be tested. The results of such an experiment are given in Fig. 4. It can be seen that, at least in the beginning, there actually was an acceleration of the fall in the derooted plants.

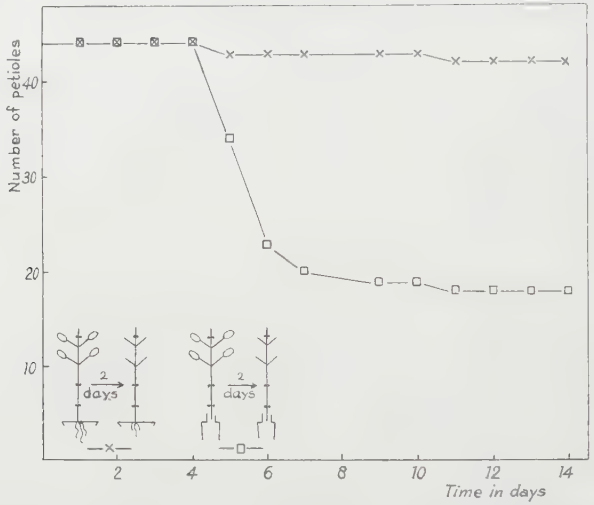

Fig. 4. The influence of derooting on abscission of petioles.

It was tried whether in the plants with roots but without leafs the fall of the petioles could be induced afterwards by putting IAA (2.10^{-3} g/cm^3) on the cut surface of the decapitated plant. For this purpose in two sets of plants the tip and all the leaves and axillary

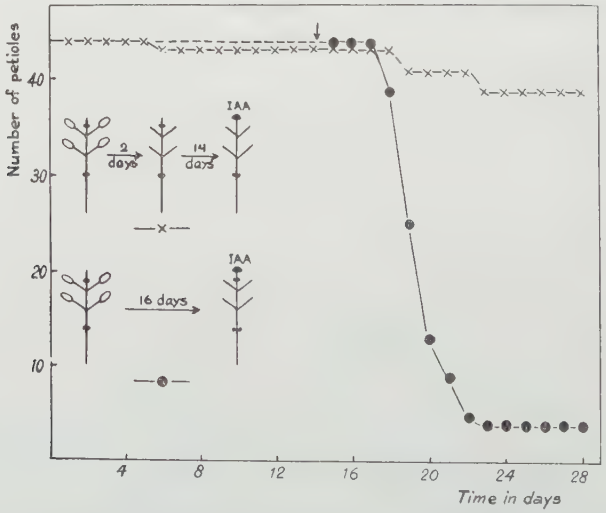

Fig. 5. The influence of IAA, applied proximal of the abscission zone, on the fall of petioles in wholly debladed plants. *a*. Application of IAA at the moment at which the petioles tested are debladed. (●) *b*. Application of IAA 14 days after deblading the petioles tested. (×)

buds were removed except the leaf pairs 2 and 3. After two days the leaf blades of leafpairs 2 and 3 of one set of plants were cut off. At the end of 14 days all plants received a treatment with IAA paste and at the same time the leaf blades of the leaf pairs 2 and 3 of the second set of plants were taken away. As can be seen from Fig. 5

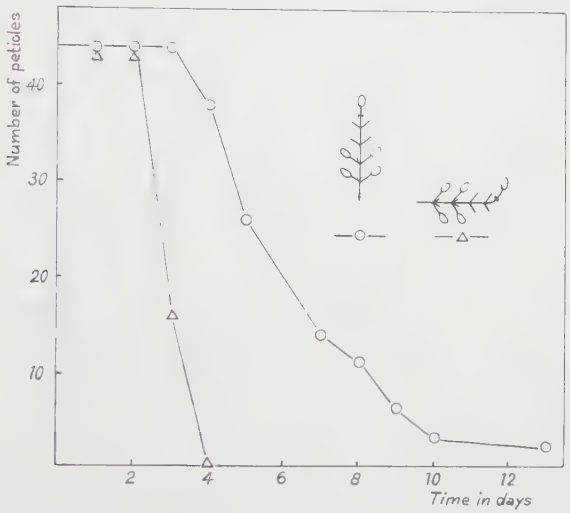

Fig. 6. The accelerated abscission of petioles in plants placed horizontally.

the IAA on the top had only a clear effect if the leaf blades were removed at the same time, it could only induce to a slight degree the fall of the petioles that had previously been debladed. These experiments will be discussed below.

B. Experiments with plants in a horizontal position

a. Influence of the position of a plant on the abscission of petioles

The findings, reported in the foregoing section, which indicate that the presence of a growing tip accelerates the fall of debladed petioles seem to fit the experience of fruit growers that a rapid growth of a tree results in an increased fall of young fruits. As in practice bending the branch in a horizontal position is done with the object of reducing the growth of that branch it was expected that putting a *Coleus* plant in a horizontal position might have a retarding influence, if any, on abscission of debladed petioles. However, the experiments showed that the reverse was true. The fall of petioles was compared in normal vertical plants and plants that one day before the experiment had been placed in a horizontal position, only allowing the tip to grow upwards. There proved to be a marked acceleration of the fall in the horizontal plants (Fig. 6). It was thought that the horizontal position of the roots might influence the results. Experiments with bent plants, where the pot remained in a normal position gave, however, the same results. It was tried whether the acceleration

effect could be obtained with decapitated plants. This proved to be the case, provided decapitation and deblading of the test-petioles took place at the same time. If, however, there was an interval of two days between decapitation and deblading of the test-petioles, the effect was much less marked or had disappeared. (Fig. 7).

The results obtained with plants with apical buds could be reproduced with plants in which the tip had been substituted immediately

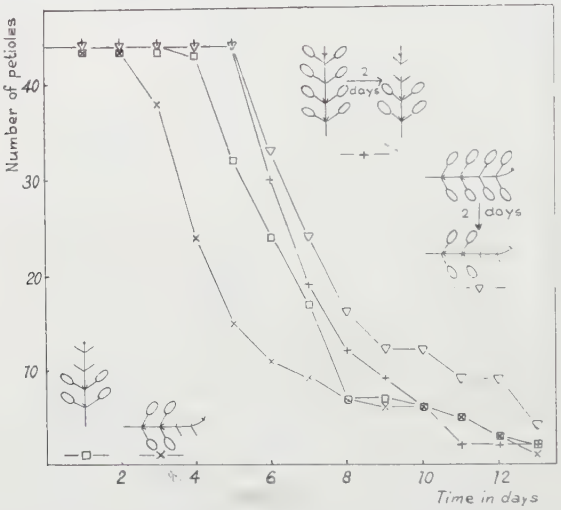

Fig. 7. The effect of decapitation on the abscission of petioles in horizontal plants. *a.* Deblading of the test petioles and decapitation at the same time (□, ×) *b.* Deblading of the test petioles two days after decapitation. (+, ▽)

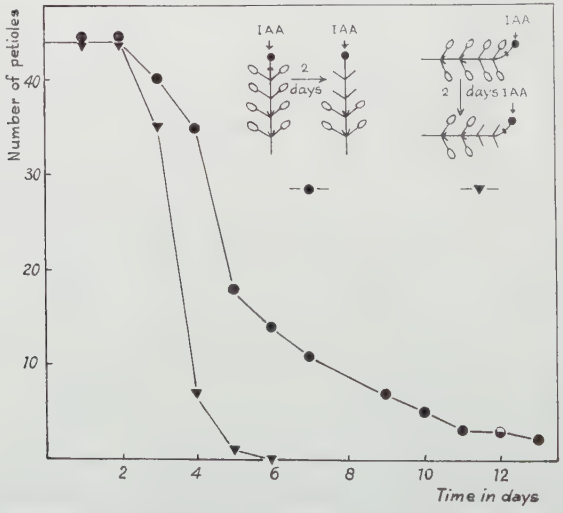

Fig. 8. The accelerated abscission of petioles in horizontal plants in which the tip has been substituted by IAA.

after decapitation by IAA (2.10^{-3} g/cm³). (Fig. 8). In a preliminary experiment it was tried whether the effect in plants that had been decapitated some days in advance, could be restored by means of putting IAA on the cut surface of the tip. To this end a set of plants was laid horizontally and decapitated after one day (at the same time the axillary buds of the leaf pairs 2 and 3 and the first leaf pair with its axillary buds were cut off). After 8 days the lanolin-water emulsion, which had been applied to the cut surface of the tip, was replaced by IAA (2.10^{-3} g/cm³) and the leaf pairs 2 and 3 were debladed. Vertical plants, treated in the same way, served as controls. There was a clearly accelerated abscission in the horizontal plants.

It is clear from these experiments that for the acceleration of petiole abscission in a horizontal position the presence of some substance, coming from the tip, is necessary. This substance can be replaced by IAA and so it is very likely that the growth substance, produced by the tip, is needed for producing the effect. The acceleration of abscission in horizontal decapitated plants that have been debladed immediately after decapitation may be ascribed to the presence of some residual growth substance, the amount of which diminishes gradually. In later experiments, made in the middle of the winter, the interval of two days after decapitation did not suffice to prevent the accelerating effect and a five days interval was used. Probably this has to be ascribed to the reduced light intensity in the greenhouse.

The results obtained with *Coleus* were checked with leaves of apple seedlings. From two sets of six seedlings, as nearly as possible in identical pairs, six leaf blades per plant were removed, leaving about four leaves at the tip. One set was placed horizontally. The experiments were made at 26° C. The results (Fig. 9) show, that the fall-accelerating effect in horizontal plants could also be observed in apple seedlings.

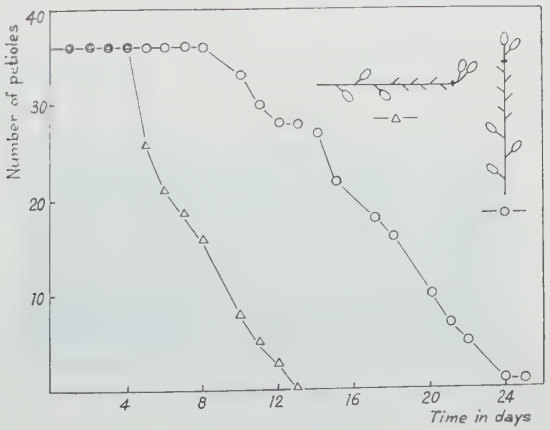

Fig. 9. The accelerated abscission of petioles in horizontal apple seedlings, compared to vertical ones.

b. Preliminary experiments concerning the cause of the accelerated abscission in horizontal plants

It was supposed that the results reported in the previous section were due to the lateral action of gravity. If plants are laid horizontally the longitudinal component of gravity is eliminated and the transversal component becomes as great as possible, while in vertical plants matters are just reversed. There were some indications that the transversal component played some role, as in general the petioles on the lower side of the horizontal plants did fall more readily than the petioles on the upper side. However, the difference could not account for the whole of the acceleration effect and besides might be due to shading, as according to JACOBS (1955) shading of petioles may accelerate their abscission. In order to distinguish between the action of transversal and longitudinal components, a set of plants was placed on a clinostat [1] with horizontal axis (one rotation in about four hours). The plants were rotated for one day before the leaf blades were removed. (In these experiments plants with 3–4 full-grown leaf pairs were used and the petioles of the leaf pairs 1 and 2 served for testing, as working with large plants on the clinostat was rather difficult). As controls identical sets of horizontal and vertical plants, treated in the same way, were used. It appeared that in the rotated plants only a slight acceleration or none could be observed. It may be concluded, therefore, that the longitudinal component of gravity is hardly or not the causal factor in accelerating petiole abscission. Yet it is not certain that the transversal component of gravity induces the effect, as the one-sided action of light may play some part. Some experiments were made with one-sided and all-sided illuminated vertical plants. One set of plants was placed before a group of four TL tubes (Philips no. 33, 40 Watt, distance from the plants 80 cm), while a second set rotated before the same light source on a clinostat with vertical axis. No significant difference in the rate of abscission of petioles in the two groups was observed. Hence we may conclude that one-sided illumination is not the causal factor in accelerating abscission in horizontal plants, and that this effect is very likely induced, at least for the greater part, by the transversal component of gravity.

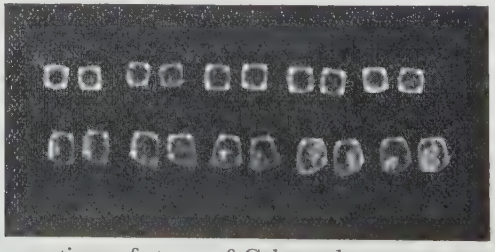

Fig. 10. Transverse sections of stems of Coleus plants grown vertically (*a*) and horizontally (*b*) during 12 days.

[1] The clinostat was kindly put at our disposal by the Botanical Laboratory of the State University at Utrecht.

Another observation was made which may throw some light upon the explanation of the phenomena described. In Fig. 10 some transverse sections are shown of stems of normally grown vertical plants and comparable sections of stems with the same initial dimensions from plants that had been lying horizontally for twelve days. In the latter case there is a marked increase in thickness of the stem, at least on the lower side. As will be discussed in the next section this increased growth may have some causal relation to the accelerated fall of petioles.

Discussion

Addicot and Lynch (1955) gave a review of the existing theories concerning abscission in which they come to the conclusion that the auxin gradient theory (Addicot *et al.*, 1955*a*) can offer a satisfactory explanation of the results of former observations and experiments concerning abscission. The theory proposes that onset and rate of abscission are regulated by the auxin gradient across the abscission zone, abscission only occurring if the normal gradient (high on the distal side and low on the proximal side) disappears or becomes reversed. In general the results of our experiments, reported in part A, support this supposition. However, there are some facts that do not agree with the theory in the form as it was proposed by addicot *et al.* In the experiments with wholly debladed plants a low auxin content on both sides if the abscission zone may be accepted, yet there occurs no or nearly no abscission. Besides, if after a number of days auxin is put on the proximal side of the abscission zone, there is still little abscission (cf. Fig. 5) and yet in this case the auxin gradient can be expected to be reversed.

Addicot and Lynch in their review (1955) mention the possibility that auxin may have an indirect effect on abscission: on the distal side of the abscission zone it might help to maintain the flow of nutrients to the leaf, while on the proximal side it might accelerate the withdrawal of nutrients from the petiole and in this way accelerate abscission. One might go one step further and suppose that all auxin producing organs in the plant (the growing tip, leaves) are attracting the flow of nutrients in the stem, thus depriving the other organs of these nutrients and in this way accelerating their abscission. In that case the nutrients coming from the roots (among which water has to be included, cf. Portheim, 1941, Carns *et al.*, 1951) may be expected to play a role. The experiments with plants without roots might point in this direction: removal of the roots results in an accelerated fall. However, these experiments may likewise be explained by accepting a direct action of proximal auxin in the abscission zone: it might be supposed that the roots are attracting the auxin present in the stem, thus retarding the fall of the petioles. Further experiments are needed to determine whether the action of proximal auxin is direct or indirect and what is the role the roots are playing in abscission.

It may be concluded from the results mentioned that abscission experiments with explants, following the method of Addicot *et al.* (1949) should be interpreted with caution.

As to the auxin gradient theory, it seems desirable to modify this theory to some degree, for instance as follows: For abscission to occur at least two conditions have to be fulfilled: 1. A reduced auxin production on the distal side of the abscission zone. 2. A sufficient amount of auxin (or auxin induced growth) on the proximal side of the abscission zone.

In many experiments it can be seen that some of the petioles do abscise very slowly or not at all within the experimental time (*cf.* e.g. Fig. 1), which has the consequence that the curve has not the normal S-shape that might be expected. In connection with the foregoing this may be explained by supposing that at the moment at which the leaf blade is removed the supply of auxin or the growth activity of other organs on the proximal side of the abscission zone is not sufficient to induce abscission, thus retarding or preventing this proces.

Attempts were made to explain the results obtained with horizontal plants with the hypothesis mentioned above. In order to explain the accelerated fall of leaf petioles an increased production of auxin on the proximal side of the abscission side should be supposed. It might be possible that the tips of horizontal plants produce more auxin. However, if the tips of vertical and horizontal plants are replaced by the same amount of IAA, the fall-accelerating effect is still present, while the auxin "production" of the artificial tips in both sets of plants must be the same.

Another supposition might be that there is an increased growth of the tip in horizontal plants in reaction to the geotropic stimulation. In this case the tip could attract an increased amount of nutrients, thus withdrawing them from the petioles to be tested. However, it was shown by Cholodny (1929) that during the time the geotropic reaction takes place the position of the growing stem has no influence on its growth, nor on its production of growth substance.

It is generally accepted in practice that in the long run the growth in horizontal branches diminishes; this phenomenon was described by Münch (1940). In literature only very little additional information could be found about the influence of the position of an organ on its growth in length. Noll (1900) mentions a growth inhibition of horizontally growing roots from *Lupinus*, *Pisum*, *Vicia* and *Phaseolus*, compared with vertically growing ones. Bennet–Clark and Ball (1950) find indications that rhizomes of *Aegopodium podagraria* grow faster in a vertical downward position than in a horizontal position, the latter again growing faster than rhizomes in a vertical upward position. They suppose this to be caused by the action of the longitudinal component of gravity. Cholodny (1932) does not find any effect of the position of roots upon their growth. Except the experiments mentioned of Cholodny (1929) no further information on the growth in length of horizontal stems could be found. However, some other growth phenomena in horizontal branches are known. Firstly, it is often observed that axillary buds grow out if branches of fruit trees are bent horizontally (Gardner, 1925). Secondly, Hofmeister

(1867) already pointed out that there is an increased growth in thickness on the upper side of the horizontal branch in a large number of trees. The effect was studied extensively by MÜNCH (1938). PRIESTLY and TONG (1927) described the phenomenon for apple trees; in our experiments it was also noticed in the horizontal apple seedlings in which we had observed the accelerated fall of petioles. PRIESTLY and TONG ascribe this effect to an influence of gravity on cambial activity. As has been shown in the experimental part, in our experiments with *Coleus* there was also an increased growth in thickness in horizontal plants, though in this case on the lower side. It may be supposed that this growth induces an increased auxin production or that certain nutrients are attracted during the increased growth and are thus withdrawn from the abscission zone. Anatomical investigations about this increased growth have not yet been performed.

The experiments with plants on a clinostat suggest that the accelerated abscission in horizontal plants is induced, at least for the greater part, by the action of the transversal component of gravity. It remains to be investigated whether the small fall-accelerating effect, observed in some experiments with plants rotated on a clinostat with horizontal axis, is a real one or will have to be ascribed to technical imperfections. The mechanism of the action of gravity causing increased growth in thickness and, probably via this phenomenon, accelerated fall of petioles, remains obscure.

In the introduction it was pointed out that these experiments were the consequence of practical problems in fruit growing. In the light of the results of these and other investigations, an increased fruit fall in fast growing trees may be explicable, as in these trees a high auxin production or a strong attraction of nutrients by the growing organs may be expected. As regards the bending of branches in a horizontal position, one will have to reckon with the possibility that this treatment influences the fall of fruits. Yet it seems risky, at least for the present, to apply the results of these experiments directly to apple fruits. It should be kept in mind that the apple fruits are normally situated on short side shoots of the branch that is bent downwards and the influence of bending these branches on abscission of the fruits cannot yet be predicted. Further experimentation seems highly desirable.

ADDICOT and LYNCH (1955) point to the relation of abscission phenomena to other ones, for instance the growth of lateral buds. This may be extended to the whole problem of correlative inhibition (bending of branches causes the lateral buds to grow out) and even of flower formation (bending of branched is generally accepted to induce flowerbud formation in fruit trees). Progress made in one of these fields may directly be applicable to the other ones.

SUMMARY

1. Experiments with various deblading patterns in *Coleus* do not confirm in all respects the auxin gradient theory about abscission. Indications are obtained that in order to render abscission possible there should be a sufficient auxin production (or auxin induced growth) on the proximal side of the abscission zone.

2. Placing the plants in a horizontal position induces an accelerated fall of

debladed petioles. This effect is only clear if a certain quantity of auxin (or auxin induced growth) is present at the proximal side of the abscission zone. Some preliminary experiments on the clinostat suggest that the accelerated fall is induced, at least for the greater part, by the transversal component of gravity. The effect is supposed to be connected with an increased growth in thickness in horizontal stems.

The consequences of the results for practical fruit growing are discussed.

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A DELIMITATION OF MAMMEA L.

BY

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I. Classification of Mammea L. and Ochrocarpos Thou. (Guttif.)

Linnaeus published the genus *Mammea* in 1754. He derived the name from Mamei or Mamey, a West Indian vernacular name for *Mammea americana* L., as recorded by Plumier (1703). The oldest reference in print was probably made by Oviedo (1535).

Linnaeus described the genus:

„Mammea. † Mamei Plum. 4. Cal. Perianthium diphyllum: foliolis ovatis, concavis, parvis, deciduis. Cor. Petala quatuor, subrotunda, concava, patentia, majora calyce. Stam. Filamenta plurima, simplicia, subulata. Antherae subrotundae. Pist. Germen subrotundum. Stylus conicus, longitudine staminum. Stigma simplex, persistens. Per. Bacca carnosa, maxima, stylo acuminata, sphaerica, unilocularis. Sem. quatuor (vel unum), callosa, subovata".

Two species were referred to the genus, *M. americana* L. and *M. asiatica* L., both already appearing in Species Plantarum (1753).

Miers (1875) declared that *M. asiatica* L. was wrongly identified by Linn. f. with *Barringtonia speciosa* Linn. f. from which a general confusion arose among the 19th century authors.

Knuth, in his revision of *Barringtoniaceae* (1939) accepted Miers's views insofar, as he placed *M. asiatica* L. as synonymous with *Barringtonia asiatica* (L.) Kurz. For this reason it is best to accept *M. americana* L. as the type species (cf. also Hitchcock and Green, 1935).

A survey of literature demonstrates that after Linnaeus about 20 spp. of *Mammea* were described, the majority of which, however, were at various times transferred to *Ochrocarpos* Thouars or switched back again.

The first study of importance to the problem of delimiting *Mammea* against allied Guttiferous taxa was published by Planchon and Triana (1861). These authors recognized 7 spp. in *Mammea*.

Kostermans (1956) stated, that *Ochrocarpos* Thou. was published for the first time in A. A. du Petit-Thouars, „Histoire des Végétaux receuillis dans les isles australes d'Afrique", in 1804, for some reason deducing this from a paper by Woodward (1900). Actually, Woodward's paper contains no statement leading to this, erroneous,conclusion. The work by Du Petit-Thouars of 1804 contains no reference to

Ochrocarpos, as is shown by the copy present in the British Museum library. Neither is there in the 1806 re-issue of the same work any reference to *Ochrocarpos*. There is, however, an 1805 copy at Kew, which contains a plate to which the name *Ochrocarpos* was added (see SPRAGUE, 1934).

In 1806 the genus *Ochrocarpos* was described and validly published by A. A. DU PETIT-THOUARS. As, afterwards, the genus *Calysaccion* WIGHT (1840) has been generally declared to belong in *Ochrocarpos*, it might be asked why Planchon and Triana l.c. did not state their views regarding a possible merging of the genera *Mammea* and *Ochrocarpos*, as they accepted *Calysaccion* as part of *Mammea*. It would appear that the problem did not occur to them because *Mammea* and *Ochrocarpos* were placed by them in different tribes, *Mammea* in *Calophylleae* and *Ochrocarpos* in *Garcinieae*, following CHOISY's arrangement in DE CANDOLLE (1824).

The tribes *Calophylleae* and *Garcinieae* as proposed by CHOISY (l.c.), were accepted by PLANCHON and TRIANA (1861), but they changed their delimitation.

A comparison of their new delimitation of the tribes *Calophylleae* and *Garcinieae* shows that the only differentiating characteristic is in the embryo; whether it has thick cotyledones (*Caloph.*) or is without or with minute cotyledones (*Garc.*), and for this reason it seems warranted to infer that they took *Mammea* as having a Calophyllaceous embryo and *Ochrocarpos* as having a Garcineaceous embryo. Also, that *Calysaccion* had a Calophyllaceous embryo and, at any rate, they explicitly stated that they were unable to keep *Calysaccion* apart from *Mammea* on account of the ovarial characters which, at first, had been supposed to be suitable to separate them.

BENTHAM AND HOOKER (1862) at first followed the delimitation of *Garcinieae* and *Calophylleae* as proposed by Planchon and Triana and applied the appearance of the embryo (cotyledones) as the distinguishing character. They accordingly placed *Calysaccion* as synonymous with *Mammea*. However, in 1867, in the Addenda and Corrigenda to vol. I of the Genera they changed their view, shifting *Calysaccion* to the synonymy of *Ochrocarpos* Thouars.

They declared that *Calysaccion* and *Ochrocarpos* were certainly to be united having both the same essential characters viz the calyx of *Mammea* and an embryo and stigma as in *Garcinieae*. They added that the spp. of *Mammea* of the Old World, recognized by Planchon and Triana had to be included in *Ochrocarpos*. It is evident, that to distinguish *Mammea* and *Ochrocarpos* the only characters thought to be at their disposal were: 1. a difference in the embryo (cotyledons), 2. a difference in the style or stigma, and 3. the distribution. The geographical argument (3), of course, only holds, if the other characters appear to be constant differences. As regards the 2nd differing character, Bentham and Hooker admitted that they had no certainty while remarking that various authors described the style differently.

As regards the embryo, supposed to show the first difference referred to by Bentham and Hooker, ENGLER (1895 and 1925) suggested that

the fleshy part of the embryo of *Ochrocarpos*, which had been regarded as the "tigella" (hence its classification in *Garcinieae*), was actually the result of connate fleshy cotyledons and he therefore removed *Ochrocarpos* to *Calophylleae*.

Pierre (1883), who was in a position to examine fresh materials, had supplied the data on which Engler's opinion was based.

Brandza (1908) again investigated the embryo's of some Guttiferous genera, among them *Mammea* and *Ochrocarpos*. He investigated only *M. americana* L. and *O. siamensis* T. Anders. and concluded that *Ochrocarpos* ought to be placed in *Calophylleae* on account of the characters of the embryo (large, fleshy cotyledons). He supported therefore, the view of Engler and also of Van Tieghem who had arrived at the same conclusion. Van Tieghem (1885) had found the resin ducts distributed in the root and bark of *O. siamensis* in the manner characteristic of *Calophylleae*.

As a result of his anatomical research Van Tieghem stated: „il en faut conclure que ce genre (*Ochrocarpos*) appartient à la tribu des Calophyllées, non à celle des Garciniées".

It might be objected that both Brandza and Van Tieghem investigated only a single, and the same, species of *Ochrocarpos*: *O. siamensis* T. Anders. Obviously, it would be preferable to have a wider range of species investigated but the opinion of Perrier de la Bâthie (1948) might be added to their conclusion. As a result of his research in Madagascar species of *Ochrocarpos*, after having examined the embryo's of many spp., Perrier de la Bâthie denied all value to embryonal characters if it were desired to place the genus *Ochrocarpos* into *Calophylleae* as the embryo's appeared to possess in the various spp. all characteristics ascribed to *Calophylleae* or *Garcinieae*. His evidence and opinion therefore do not contradict the view that *Mammea* and *Ochrocarpos* belong in the same tribe, whatever its name should be. In Perrier de la Bâthie's revision of *Guttiferae* for Madagascar (1950), no tribes are indicated.

It appears, therefore, that all the evidence found so far goes to show that the first essential character employed in separating *Mammea* and *Ochrocarpos* by Bentham and Hooker, as referred to above, does not hold, like the other characters employed.

As regards the wood anatomical evidence, Metcalfe and Chalk (1950) appear to have knowledge only of *M. africana*. They find it different from all other genera of *Calophylloideae* in having diffuse parenchyma.

Mr. C. H. Japing, of the Div. of Forest Exploitation and Forest Economics in the Institute of Forestry at Wageningen, was kind enough to investigate a specimen of wood of *M. americana* L., and he informed me that the anatomy, especially as regards diffuse parenchyma strikingly resembled that of *M. africana* Sabine. This anatomical character supports Van Tieghem's results and stresses both the affinity of *M. americana* L. and *M. africana* Sabine and their isolated position in *Guttiferae*.

A. Engler (1925) wrote the most recent general revision of *Gutti-*

ferae. In his key he placed *Mammea* and *Ochrocarpos* side by side. *Mammea* is characterized by a 4-2 loculed ovary, the locules containing in total 4 ovulae. The flower is axillary. The stigma is 2-4-lobed. *Mammea* occurs in tropical America and Africa. Engler opposed *Ochrocarpos* by means of the following characters: 2-loculed ovary, each locule with 2 ovulae. Flowers in fascicles. Stigma peltate. Distributed in the tropics of the Old World.

These characters are for differential purposes of no value. From Engler's description of the genera no new possibilities of separating *Mammea* and *Ochrocarpos* appear.

The suggested difference in number of loculi in the ovary is, actually, nonexistent. A 4-loculed ovary appears to be brought about by a retarded emerging of a (mostly only partial) sept (cf. Viguier and Humbert (1914). Perrier de la Bâthie (1950), also, described *Ochrocarpos* as occurring in Madagascar as having an ovary with 4 uniovulate loculi, "complètes ou incomplètes". There is, evidently, no difference here.

Engler stated that the female flower of *Mammea* was solitary (l.c., p. 190), whereas he described *Ochrocarpos* (in the key and in the description) as having fascicled flowers. This also is no real difference as e.g. *O. eugenioides* (Pl. and Tr.) Vesque and *O. punctatus* H. Perr. have solitary female flowers. All *Ochrocarpos* spp. have axillary flowers, though this character is used by Engler to characterize *Mammea* (in the key).

As regards the suggested difference in the appearance of the stigma (*Mammea*: 2-4-lobed, *Ochrocarpos*: peltate), it suffices to examine Engler's plate illustrating the genus *Ochrocarpos* (l.c. p. 193) where a perfectly 4-lobed stigma is figured.

Staner (1934) also considered the problem of separating the genera *Mammea* and *Ochrocarpos*. He limited his investigations to taxa occurring in the Belgian Congo which implies that he considered a single species, viz *Mammea africana* Sabine (other described *Mammea*'s for this region proved to be conspecific). He found that the flowers of *M. africana* Don (sic) might occur in fascicles or be solitary. Staner tried to separate *Mammea* and *Ochrocarpos* on the presence (O.) or absence (M.) of a so-called "boutonnière", a smal gap present between the cotyledons becoming visible in a transverse section of the embryo.

Whatever the meaning of this observation may be, no later author has attached any importance to it and Staner himself finished by stating: "Il est même vraisemblable qu'une étude complète des deux genres amènerait à ne plus considérer *Ochrocarpos* que comme un sous-genre de *Mammea*".

Kostermans (1956), in his above-mentioned recent paper proposed to extend the limits of *Mammea* considerably. He wished to characterize *Ochrocarpos* by fascicled stamens and non-areolate leaves, and *Mammea* by free or nearly free stamens and, "areolate" or distinctly reticulate leaves. This implies that the section *Euochrocarpos* Vig. et Humb., as regards the Madagascar spp. is added to *Mammea* and also all E Asia

and S Pacific spp. sofar accepted as *Ochrocarpos*. The genus *Ochrocarpos* is limited to Madagascar and *Mammea* also occurs on that island, according to Kostermans.

As a result of the survey of literature given above and my own study I am in favour of widening the limits of *Mammea* so as to include *Ochrocarpos* entirely and I, therefore, am not prepared to maintain the latter genus, not even in the restricted sense proposed by Kostermans, which I find untenable for the following reasons.

1. A new combination by KOSTERMANS (1956) is *Mammea glaucifolia* (H. Perr.) Kosterm. The flowers of this species are unknown and so are the characters of the stamens. The leaves are leathery, the nerves are certainly not characteristic of *Mammea* sensu Kosterm., but suggest strongly the kind of nervation commonly met with in *Garcinia*. (cf. Ursch 143, type, in Herb. Mus. Paris).

2. The same question can be raised for *Mammea cerasifer* (H. Perr.) Kosterm., from which I saw the type (Decary 5659, Herb. Mus. Paris). Again the flowers are unknown, but the nervation is without doubt that of *Ochrocarpos* sensu Kosterm. PERRIER DE LA BÂTHIE (1948) alluded to the secretory canals which were more oblique than usual and crossing more or less the secondary nerves. This and the drawing in the Flore de Madagascar (1950) may have led Kostermans to assume that the leaves would match what he believes to be characteristic for *Mammea* L. but the type material has leaves which, as to their nervation, are indistinguishable from *Garcinia*.

3. No mention is made by Kostermans of *O. sessiliflorus* Vesque (in DC., 1893). The stamens in this species are free (PERRIER, 1950) and so it should belong in *Mammea* according to Kostermans. The leaves, however, are as regards the nerves, uncertain to place, perhaps slightly more like *Garcinia* sensu Kostermans, but ultimately it remains largely a matter of taste (cf. the syntype Martin No. 3, "Madagascaria" in Herbier Delessert, Genève). It is again a transitional taxon, if considered in the light of Kostermans's suggestions.

4. *O. decaryanus* H. Perr. has an "androcée en colonne cylindrique centrale" and so is referable to *Orchocarpos* sensu Kosterm. Perrier's (1950) description leaves no doubt that the leafcharacters are like *Mammea* sensu Kosterm., which could be confirmed by examination of the type (Decary 5161, Herb. Mus. Paris) and of Alleizette s.n. X-1905, Analamazaotra (Herb. Leyden).

5. *O. bongo* Vig. et Humb. (1914) of which I examined the type (Vig. et Humb. 849 in Herbier Delessert, Genève) shows leaves which are obviously, as regards the nerves, referable to *Ochrocarpos* sensu Kosterm. However, Kostermans refers the species to *Mammea* (l.c. p. 12), while admitting that: "the stamens.... are grown together more at the base than is found in the other known species", which stresses the occurrence of intermediate stages between united and free stamens.

The picture of the stamens of *O. bongo* Vig. et Humb. presented by PERRIER (1950) clearly demonstrates an intermediate position between free- and fascicled (connate) stamens. This again is a transitional taxon.

In conclusion I feel it is justified to say that there is no sharp demarcation as regards the stamens, whether they are free, partly connate or fascicled among the species ascribed either to *Mammea* or *Ochrocarpos*. Secondly, there is also no clear demarcation as regards an areolate ("reticulate") nervation, as found in *Mammea* L., and the nerves as usually seen in the section *Paragarcinia* Baillon (1876), (cf. Kostermans l.c. p. 11); on examining a number of specimens and trying to sort them out, one is very soon entirely at a loss to separate them on the strength of leaf-nervation.

Both these characters of the leaves and the stamens, seen separately, appear to be present in various intermediate degrees between two extremes. It might be suggested that, though being unsatisfactory as characteristics by themselves they could be correlated to such an extend that a satisfactory systematy could be based on this correlation. Although they are somewhat correlated — as was correctly observed by Kostermans — this correlation is certainly too laxly maintained to be decisive in separating *Mammea* and *Ochrocarpos* (cf. notes 1-5) and already led to unsatisfactory decisions as regards the placing of species in the supposed genera by the proposer himself.

A final point to be made is that Kostermans (l.c.) separated *O. perrieri* Vig. et Humb. and *O. punctatus* H. Perr. from both *Mammea* and *Ochrocarpos*, on account of dehiscent fruits. For that reason these species "apparently belong to another genus" (l.c. p. 15). To me, this seems a suggestion to be taken up with caution as in many, perhaps the majority, of species referred to *Ochrocarpos* or *Mammea* the characters of the (mature) fruit are unknown.

Apart from this, in *O. perrieri* Vig. et Humb. the stamens are entirely connate (cf. Perrier 1950), and in *O. punctatus* H. Perr. free (l.c. p. 74), which implies that one of the two essentials for distinguishing *Mammea* and *Ochrocarpos*, adopted by Kostermans, is already of no value in the most nearly allied taxon, the suggested new genus.

I conclude that the entire genus *Ochrocarpos* is to be united with *Mammea* L., there being no consistent characters or correlation of characters by which they could be distinguished.

It might further be pointed out that the two-valved calyx (resulting from fissure) is found in *Guttiferae* only in *Mammea* L. (and *Ochrocarpos* Thou.). There is no species known as an exception to this rule and so this peculiarity is an excellent character to keep a taxon apart in *Guttiferae*. Also, the wood anatomy, as far as is known, stresses a well-demarcated position in *Guttiferae* for a taxon composed of *Mammea* L. and *Ochrocarpos* Thou.

II. Mammea described

A Guttiferous genus. Leaves: Blade coriaceous to chartaceous, often with pellucid glands or pellucid secretory canals, pinnately nerved, often reticulate, the reticulations lax and open to very densely crowded.

Flowers polygamous, sometimes dioecious, solitary or more numerous in axillary, short, cymes or apparently indefinite inflorescences, often cauliflorous. Calyx in bud without any trace of separate valves, at

anthesis tearing into two parts (very rarely into 3 parts), more or less persistent in fruit. Petals usually 4, sometimes 5 or 6, free, caducous. Stamens in male flowers numerous, free, at the base connate, or nearly entirely connate (forming a column) or in 4-5 phalanges; in the female or hermaphroditic flowers less numerous, free or only at the base shortly connate, or staminodial.

Ovary generally with 4 ovules and 2-4- (or very rarely many) celled (septs complete or not). Style absent or short. Stigma peltate, 2-4-lobed or rather irregularly denticulate.

Fruit baccate or drupaceous, indehiscent or, perhaps, very rarely dehiscent by 2 or 3 valves. Seeds 1 to 4, very rarely more.

Evergreen trees or shrubs in rain forests, or in deciduous forests, containing abundant yellow or white latex. Flowers white or pink. In Madagascar spp. the closed calyx is mostly apiculate.

Distribution: Circumtropical. America: West-Indies, Central America, northern part of S America. Africa: Tropical (West) Africa between about 10° N.L. and 10° S.L., and Tropical East Africa very locally (Usambaras). Madagascar. Asia: SE Asia and into the SW Pacific.

Note: The genus is generally found in the lowland, but in Madagascar also frequent in the mountains, up to about 2400 m. In East Africa it was found at 2000 m. At present 38 spp. are referable to *Mammea*, the majority already published as such; some spp. of *Ochrocarpos* are to be renamed in accordance with the systematy proposed here but I have refrained from publishing the required new combinations pending a monograph dealing with the species in detail (cf. Code, Rec. 17A, and Kostermans l.c. p. 11).

The record of a finding locality in E Africa at 2000 m is based on a specimen present in the Kew Herbarium (R. B. Drummond and J. H. Hemsley 2727). The collectors found it in the Western Shagai Forest, Usambaras, 2000 m, dominant in certain small areas, otherwise scattered in Ocotea-Podocarpus forest.

The fruits of this specimen are very much smoother than the warty and rough skinned fruits known from the type locality of *M. africana* Sabine (Sierra Leone, Kew Herb., or also de Wit, coll. no. 896, Bot. Gard. Bingerville, Herb. Wageningen) and it deserves further research whether a new species of *Mammea* is at hand and also whether there could be some connection with ENGLER's (1925) remark on the occurrence of *Mammea* in E Africa and the cultivation of a species in the Botanic Garden at Victoria.

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Naturelle, Laboratoire de Phanérogamie); Salisbury (Queen Victoria Memorial Museum); Utrecht (Botanical Museum and Herbarium).

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GROWTH OF POLLEN TUBES IN VITRO AND THEIR REACTION ON POTENTIAL DIFFERENCES

BY

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I. Culture of pollen in vitro

About a century ago the first attempts were made to germinate pollen grains in vitro. At present several methods have been developed which facilitate the germination of pollen grains and the growth of the pollen tubes emerging from them, in an artificial medium.

The moist chamber with the hanging drop, well known in microscopical technics, is now commonly used. The pollen grains and the tubes produced here can thus be continuously examined. Instead of a moist chamber for one drop of culture solution, a petri dish in which more drops hang down from the cover is used, so as to observe a series of cultures under the same circumstances.

Pollen tubes should grow many centimeters, for which they need more space than there is in one drop of culture solution, so we successfully used culture tubes containing 4 ml sterilized medium plugged with non-absorbing cotton, well known in microbiological technics. In this way not only sterile or semi-sterile cultures can be obtained, and, besides, the large volume of culture medium is a more stable medium than a little drop.

Pollen grains of many species of plants germinate in pure water but those of other species are even damaged in it. Moreover, when pollen grains are surrounded by a liquid medium they lack oxygen, which is necessary for the intensive respiration during the germination period (van Tieghem 1869, Okumuki 1932, Linskens 1955). Therefore, agar is usually added to the culture medium; so the pollen grains are sown on a surface. The addition of gelatine has been abandoned, and silica gel (Johnson 1943) and calciumpectate gel, as we established in preliminary investigations, did not give satisfactory results. In most cases 1 to 2 % of agar added to the medium is sufficient. It is necessary to wash out the agar thoroughly with distilled water before mixing it with the culture solution, otherwise the germination of the pollen grains will be severely influenced.

Many investigations have shown a favourable effect of carbohydrates on the germination of pollen grains as well as on the growth of the pollen tube. These substances partly function as nutrient and partly they stabilize the osmotic properties of the medium.

The sucrose commonly used may be replaced by one of a few other carbohydrates as glucose (Miyoshi 1894 b), sometimes fructose (Burck 1900, Bair 1941) and even lactose (Bishop 1949). The optimum concentration of the sugar in the medium must be determined for each species of pollen, but it has for every species a wide range. Often there is an optimum at about 10 % sucrose.

The germination of pollen is very sensible for the pH of the medium. There is an optimum at about pH 5, but individual differences occur. The maximum percentage of germination is not always obtained at the same pH as that at which the pollen tubes reach their greatest length (vom Berg 1930). The addition to the culture medium of organic acids such as 0.01 % malic acid or citric acid had some favourable effect especially with the pollen of *Ericaceae* (Molisch 1893, Lidforss 1896, Jost 1907, Kühlwein 1937).

A favourable effect was shown by addition of 0.01 % to 0.001 % of boric acid as well on the germination as on the growth of the pollen tubes of *Nymphaeaceae*, *Liliaceae* and *Amaryllidaceae* (Schmucker 1933, 1935, Ehlers 1951). It is probable, however, that the function of boric acid is not only to bring about the acid reaction, but there may also be an influence as a trace element, for the effect of boron on the pollen tube growth seems to be dependent on the boron contents of the plant (Visser 1955).

Other trace elements Mn and Zn are reported to have some beneficial effects (Huang 1948). Most salts, however, proved to be injurious to the pollen tube growth except perhaps calcium salts at 5 to 25 milliaequivalents (Okumuki 1932, Bungenberg 1934).

Pollen tubes are grown at temperatures which range from 20° C to 30° C with an optimum for most plants at about 25° C. Usually temperatures under 15° C and over 35° C have growth-retarding effects. There is some ecological adaptation, so pollen grains of tropical plants need more heat than plants from temporate regions, just as waterplants possess more water-resistant pollen than desert plants.

II. The influences on the direction of pollen tube growth

One of the problems in the pollen tube growth was, how did the pollen tube find its way into the stylar canal and to the ovules in the ovary.

An attracting influence of the stigma on the growing pollen tube in vitro has been indicated in the case of several plants such as *Primula* (Correns 1889), *Amaryllis*, *Clivia* (Molisch 1893) and *Narcissus* (Brink 1924). Detailed research on 36 species of plants showed attractivity of the stigma on the pollen tubes of 10 species, among which *Hippeastrum*, *Lilium*, *Narcissus* and *Antirrhinum*. The style and the stigma attracted the pollen tubes only in young and mature condition whereas the placenta and the ovules did so in every case, but the distance was never more than 1 to 1.5 mm (Tsung 1949).

Capillaries filled with sucrose are said to attract pollen tubes of *Digitalis* and *Oenothera* at a distance of about 1 mm (Miyoshi 1894 b), but this chemotropism is strongly influenced by the culture medium

(Lidforss 1909). Other chemical substances failed to attract the pollen tubes (Tsung 1949).

Effects of gravitation and influence of light on the direction of the pollen tube growth could not be established (Kny 1881, Miyoshi 1894 a).

The influence of potential differences upon the growth of the pollen tube is not at all clear yet.

The pollen tubes of *Impatiens* grew to the anode when sown on agar between two platinum electrodes with a direct current of about 0.03 mA (Wulff 1935). We calculated that there must be a potential difference of about 1.3 volts per cm. The length of the pollen tubes was less than 1 mm, whereas about 4 mm is reached in vitro (Ehlers 1951).

Contrary to this the pollen tubes of *Vinca rosea* grow for 80 % in the direction of the cathode at a direct current of 0.55 mA per mm^2 (Marsh and Beams 1945). The potential difference was perhaps more than 10 volts per cm and in this case the pollen tubes reached only a length of 0.1 to 0.5 mm, whereas in vitro they usually grow up to 10 mm (Bobilioff-Preiser 1917).

If pollen tubes react on potential differences one would expect that potential differences should be shown in the style of the flowers.

Between several parts of a plant potential differences even up to 200 millivolts have been established. They are attributed to diffusion processes in the cells or to oxido-reduction systems acting in the tissues.

The potential differences measured depend on the electrodes used, hence the difficulty of definitively establishing the value of such potential differences (Umrath 1928). These measurements are further complicated by electrical effects occurring at the surface of wounds by which the injured place becomes negative with respect to the uninjured part. The maximum difference is reached a few minutes after the injury took place, and then it gradually fades away (Kümmel 1930).

The style of *Narcissus* and *Primula* has a preferential conductivity. When a current of about 0.0003 mA was run form the style to the ovary the conductivity was greater than when the current was run in the opposite direction (Guha 1927). So the resistance for a current running in the direction from the ovary to the stigma is greater than when conducted in the opposite direction and from this we expect that there may exist a potential difference in which the stigma is positive and the ovary negative. If this is so, the pollen tubes under natural circumstances grow to the negative part of the carpel.

Thus we were interested to know what potential differences could be shown in the styles of the flower and how the pollen tubes reacted upon potential differences in vitro.

III. Plant material and methods used in our investigations

The plant material we used, *Narcissus Pseudonarcissus* L. var. *Golden Harvest*,[1]) was available throughout the year. There was little difference

[1]) Obtained from the Laboratorium voor Bloembollenonderzoek at Lisse, by kind permission of Prof. Dr. E. van Slogteren.

between pollen from forced plants and normal pollen (viz. NOHARA 1924). The pollen was gathered as soon as possible after the opening of the flowers and stored over calciumchlorid in the dark at room temperature. We used the pollen for about a month, during which time no decrease of vitality of the pollen tube growth was observed. The length of the style was about 60 mm.

The culture medium consisted of 100 ml bidistilled water, 2 g washed agar, 10 g sucrose and 10 mg boric acid puriss. The agar was obtained from agar strips which were placed in distilled water for weeks and finally in bidistilled water. We refreshed the water almost daily. The washed agar was melted on a waterbath and filtered through glasswool before use. The culture medium had a pH 5.

Each culture tube contained 4 ml medium sterilized on a waterbath for 10 minutes and then placed in a sloping position.

Inocculations of the pollen on the agar medium, as well as manipulations with electrodes were performed in as sterile a way as possible by the usual microbiological methods. The cultures were placed in the dark at 21° C and examined after about 24 hours.

For the cultivation of pollen tubes under influence of a potential difference platinum electrodes were used, 5 mm wide and 5 or 10 mm long, connected with a platinum wire of 20 cm length. The electrodes were placed in the culture tubes at a distance of 30 mm before the agar was cooled (Fig. 1).

Preliminary observations on the pollen tubes were made by means of a magnifying glass but afterwards microscopic preparations were made in the following way. The whole agar medium was pulled out

Fig. 1. Culture tube with agar medium and platinum electrodes.

of the culture tube by means of a hook with a flat end. The pollen tubes were then fixed and stained with a few drops of iodine tincture. When necessary the agar medium was cut into pieces and by means of a few drops of glycerine a cover glass was attached.

Potential differences in the flowers were measured by means of an Electrofact potentiometer, from which millivolts can be read directly within a few seconds. The electrodes used in this case were platinum needles of 0.25 mm diameter.

IV. Experiments on some factors which direct pollen tubes in their growth

a) The influence of sugar

In the sugar containing medium the pollen tubes of *Narcissus Pseudonarcissus* grow radially from the place where the pollen grains were inoculated. The length of such tubes normally ranged from 5 to 7 mm. Pollen grains sown on an agar medium containing no sugar produce pollen tubes that do not even reach half those lengths. When some grains of sugar are placed on a sugar-free medium at a distance of about 5 mm from the pollen grains, then the pollen tubes grow in all directions in the agar medium but in the direction of the sugar they are about twice as long as in the other directions.

So we could not show the existence of a real chemotropism, only an acceleration of the growth of the pollen tubes caused by sugar. This explanation is not quite the same as that suggested by Miyoshi (1894 b) and Lidforss (1909).

b) The influence of gravitation

It was observed in our cultures that the pollen tubes grew in all directions from a group of grains; they did not only grow along the surface but sought their way into the agar, too. So it was obvious that gravitation had little influence on the direction of pollen tube growth. Further, we could not establish any difference in the growth of pollen tubes, whether the culture tubes with the agar surface were placed horizontally or vertically, in both cases the pollen tubes grew radially on and in the agar medium.

Just like Kny (1881), Miyoshi (1894 a) and some others, we therefore conclude that gravitation exerts no influence on the growth of the pollen tubes.

c) Potential differences in the style

We supposed that potential differences in the style could be a guiding factor in pollen tube growth. Therefore we first investigated whether potential differences were present in the flowers of *Narcissus Pseudonarcissus*. Two platinum needles connected with the Electrofact potentiometer were introduced into parts of the flower under investigation and the potential difference read within a few seconds.

TABLE I

Potential differences in the flowers of *Narcissus Pseudonarcissus* L. var. *Golden Harvest*. Electrofact potentiometer. Electrodes: platinum needles. Three parallel measurements.

Potential difference between the top of the ovary and the corona	Potential difference between the top of the ovary and the stigma
20 millivolts	120 millivolts
70 „	120 „
10 „	120 „
Average 33 millivolts	Average 120 millivolts

The ovary proved to be negative both with regard to the pedicel and as regards the stigma and the style. There was, further, a small potential difference between the top of the ovary and the corona while a greater one existed between the top of the ovary and the stigma (Table I). Thus it is obvious that potential differences may influence pollen tube growth in the style.

It is well known that pollen tubes can grow through pieces of the style when these are cut carefully from the flower and placed in a moist chamber (Pfeffer 1886, Miyoshi 1894 a, Jost 1907, Schoch-Bodmer 1932, Straub 1946, Haeckel 1951). We were also able to establish this for the styles of *Narcissus* and *Gladiolus*.

We compared the potential differences in the style of some flowers with those of the same styles after isolating them. We found that a potential difference was left after the style had been cut (Table II).

TABLE II

Potential differences in the flowers of *Narcissus Pseudonarcissus* L. var. *Golden Harvest* compared with those in cut styles. Electrofact potentiometer. Electrodes: platinum needles. Five parallel observations.

Ovary - Corona	Ovary - Stigma	Cut Styles
20 millivolts	70 millivolts	10 millivolts
20 ,,	60 ,,	30 ,,
30 ,,	60 ,,	20 ,,
50 ,,	80 ,,	30 ,,
60 ,,	80 ,,	40 ,,
Average 36 millivolts	70 millivolts	26 millivolts

This is in agreement with the possibility that the pollen tubes in the style are influenced by potential differences. The pollen tubes of *Narcissus Pseudonarcissus* then grow to the cathodal side of the style with a small potential difference.

d) The influence of potential differences on pollen tube growth

We were interested to know whether pollen tubes are influenced in their growth in vitro by potential differences and in what way they would show their reaction. So we grew pollen tubes of *Narcissus* between two platinum electrodes placed in the agar medium culture tubes (Fig. 1). The potential differences applied ranged from 0.2 to more than 2 volts per cm. During the whole growing period the potential difference was maintained between the electrodes and examinations were made after about 20 hours.

From table III it may be seen that at a low potential difference pollen tubes do not react at all. They reach a length of about 5 to 7 mm, just like the blank tests and they grow in the same way radially in and on the agar medium.

In those cases in which a high potential difference was applied pollen tube growth was totally inhibited. Nevertheless some germination

occurs but the pollen tubes then produced are only a few times longer than the pollen grains.

At a potential difference of about 0.5 volts per cm the pollen grains were prevented to grow their tubes in the direction of the anode but they did produce their tubes rather straight in the direction of the cathode, the latter reached a length of 5 to 7 mm.

TABLE III

The influence of potential differences on the pollen tube growth. Pollen of *Narcissus Pseudonarcissus* L. var. *Golden Harvest*. Resistance of the culture medium 30 to 45 kΩ. Platinum electrodes at a distance of about 30 mm. Direct current from batteries. Avometer.

Potential differences applied in V per cm.	Current calculated in mA.	Results
0.2	0.015	Pollen tubes growing in all directions
0.4	0.03	,, ,, ,, ,, ,, ,,
0.4	0.05	Tubes only in the direction of the cathode
0.5	0.04	,, ,, ,, ,, ,, ,, ,, ,,
0.5	0.04	,, ,, ,, ,, ,, ,, ,, ,,
0.6	0.05	Short tubes in the direction of the cathode
0.6	0.09	,, ,, ,, ,, ,, ,, ,, ,,
0.7	0.09	Tubes totally inhibited; agar liquefies near cathode
1.76	0.15	,, ,, ,, ,, ,, ,, ,,

The potential difference applied to obtain these results with *Narcissus* is about half that applied to *Impatiens* (Wulff 1935) while the direction of the pollen tubes is exactly the opposite one. Further, whereas the direction of the pollen tube growth of *Narcissus* was the same as in *Vinca* (Marsh and Beams 1945), the required potential difference is much lower.

If all these results are trustworthy they suggest that there may be in various genera a specific difference in pollen tube growth with regard to potential differences.

SUMMARY

1. Pollen tube growth in vitro is accelerated by addition of sugar.
2. Gravitation has no influence whatever on the direction of growth of the pollen tubes.
3. In the flowers of *Narcissus Pseudonarcissus* L. var. *Golden Harvest* potential differences could be shown, in such a way that the ovary is negative both with respect to the pedicel and the stigma.
4. There is a small potential difference between the stigma and the top of the ovary.
5. Pollen tubes of *Narcissus* grow in vitro in the direction of the cathode and are inhibited in the direction of the anode at a potential difference of about 0.5 volt per cm.

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AIMS AND METHODS IN THE BOTANIC GARDEN "DE WOLF" OF THE STATE UNIVERSITY GRONINGEN (NETHERLANDS)

BY

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The State-University of Groningen, already in possession of a botanic garden since 1642, now owns two botanic gardens, each entirely different in character.

The old Botanic Garden,[2]) large more than 1.5 ha., is situated in the inner town behind the Botanical Laboratory. It is fitted up like the greater part of other botanic gardens, and complies with all demands usually made, viz.: having and tending a big and varied collection of plants. As a rule these are mostly seedplants: the Cryptogams are generally represented by ferns only.

The plants are grown in the usual manner. On the average they are arranged according to some system or other and provided with labels giving names and if need be further particulars. The arrangement may be in accordance with geography, aesthetics (groups of plants flowering simultaneously) or didactics (ornamental plants, utility plants, rare plants in connection with nature-conservancy) and is often of miscellaneous character in this respect.

The new botanic garden, called "de Wolf", in the village of Haren, however, was from the start and to a large extent established on quite different principles. The aim namely was, and is, dual:

I. the laying out and keeping of an arboretum, including a fruticetum; in this "de Wolf" only partially differs from other botanic gardens;
II. creating possibilities for development for as many plants possible, also for such as cannot, or only with great difficulty be grown in the ordinary way.

With which methods this aim was approached and in how far results have been attained in the botanic garden "de Wolf" in the ca. 25 years of its existence will be discussed in this paper.

Before passing on to that subject however, first some observations about the history and, roughly, about the division of the grounds, situated in Haren, a village 5 km to the south of Groningen.

Formerly it was a manorhouse, called "de Wolf", about 12.5 ha

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[2]) Further particulars about this garden in "Hortus Muntingiorum", by Che. H. Andreas. Scripta Academica Groningana 1953.

in size. In 1917 the State bought it and the idea was to have an arboretum laid out and a laboratory built for systematic botany, with a hothouse complex, that could catch a maximum of sunlight in the wide area. Later on, thoughts turned towards laying out of the garden and establishing a Biological Centre of the Groningen University there. Those plans could not be realised completely as yet.

In the winter of 1929-1930 the laying out of the garden was begun on a modest scale, to which we will return further on. The work had to be stopped during the war years 1940-1945; it was only later that it could be recommenced, to come to an end, for the moment being, in 1947.

Since 1942 the Genetical Institute, which had already for about 20 years had an experimental garden there, has been lodged in the former countryseat "de Wolf".

In 1953 the new Zoölogical Laboratory started work; it is surrounded by grounds with a.o. animal-lodgings for the use of ethological research. Further there is a small trial-plot for experimental botanical work. All this occupies about 1.5 ha. Another 1.5 ha was reserved for the future building of a Botanical Laboratory with hothouse complex and the laying out of a systematical garden for herbaceous plants and of experimental gardens. So 9.5 ha are at the disposal of the new botanic garden proper.

While considering the possibility of creating suitable conditions for growth in this botanic garden, for plants that cannot at all or only with great difficulty be grown in the usual way, such as e.g. *Pyrolaceae*, *Orchidaceae*, many alpine meadow-plants and Cryptogams like Myxomycetes, algae, scale- and leafmosses, lichens and fungi, there rose in the mind of the second author the idea of also trying to make certain types of vegetation develop.

In the laying out and fitting up of the garden, the ultimate aims were continually kept in consideration. For the aim mentioned sub II, creating possibilities for growth for as many kinds of plants possible, it was important to obtain a great variety of habitat conditions. The nature and site of the grounds made it possible to effect several differences in level, moisture status, composition and exposition of the soil.

The grounds lie on the eastern slope of the socalled Hondsrug, a glacial ridge, running from the town of Groningen, through the province of Drenthe, southeastwards. The difference in height between the western and the eastern sides of "de Wolf" consequently is about 2.75 m.

On account of the fact that the garden lies on this glacial ridge, there is a subsoil of boulderclay, on top of which is the layer for cultivation. This was heterogenously composed, which may be readily understood if one bears in mind that arable and pastural farming influenced it for many decads, probably even for some hundreds of years.

At the moment of buying, the situation of the grounds was as follows.

The manor "de Wolf", the vegetable-garden and the orchard

behind the house, and the flowergarden lying on the southside, were surrounded by a wide moat, lined with oaks, ashes, elms and limetrees; there was a subgrowth of hazel, hawthorn, guelderrose etc. Along the south- and east-sides of the wooded moat ran a ditch for drainage, to the south of which were pastures, bordered on the east-side by a ditch with willows.

For the benefit of the racehorses, formerly kept at "de Wolf", a course had been laid out along the eastern side of the grounds. Westward of this course the meadows again unfolded, surrounded and intersected by ditches, with or without willows.

On some of these pastures extra manure of moundearth was put by the former owner.

In general we may say that the cultivation-layer consisted of a very eutrophic, humous, sandy soil, with here and there great differences especially as to the humus content.

Now the fact that at "de Wolf" some difference in height and a layer of boulderclay occur, practically allowing no water to percolate, makes it possible to regulate the water effectively.

Rainwater penetrates only into the upper layers, as far as the boulderclay, and then flows down along the faint slope. If there are pits in the boulderclay or if those are purposely made, then the water remains. If these pools are continually fed artificially (vide below, pumpworks) then they retain the water all the year round; if, however, the pools only receive rainwater, then they will fall dry during the summer, entirely or partially. In the main one may say however, that even vegetations, requiring much water for their development, e.g. Shpagnum-vegetations, have sufficient water at their disposal, during summer as well. Dry spots however can also easily be effected, because the rainwater can speedily be drained off.

By building a soil-relief it was possible on the one hand to obtain layers of various thickness, pervious to water, on top of the boulderclay, and on the other hand a sufficiently thick loose culture-layer in which tree-roots could penetrate. In the deeper places the boulderclay forms the surface, in others it is loamy or humous sand which is at the surface. In some places yet another substrate is made, such as stones, stonechips, cinders and chalk.

On account of the natural conditions of the soil at "de Wolf" the differences in height caused, need not be great to be effective. The soil-relief is therefore nothing but a system of ridges and dams. The former, broad, long and sometimes flat, border the valleys; the dams are narrower and higher, and border the drainage-ditches. Some valleys have drainage, others do not. Sometimes they are pools, more or less deep, either receiving only rainwater, or principally fed by the more eutrophic, socalled depth-subsoilwater, pumped up from under the boulderclay.

The works for this purpose were erected in the highest part of the garden, by the side of the pond. This subsoilwater is brought up from 70 m depth, led into that pond and into a brook, running through nearly the whole of the Arboretum as well as through the more open

grounds. In the brook some dams have been built to prevent the uppercourse from falling dry; between the dams the current is slow, where they are lacking one can speak of a flowing stream. There plants like *Montia fontana* and species of *Callitriche*, specially dependant on flowing water, find suitable conditions. By a system of culverts as well the waterlevel desired can be maintained, while the eutrophic water is at the same time refreshed. Should the water in the pond rise too high, then it is drained off to some small pools in the promiscuous forest and from there past the meadows along a narrow bedding, finally emptying itself into above mentioned brooklet.

Owing to the great variety of conditions sketched above, combined with the methods of cultivation, yet to be described further on, many plants in many vegetations can find their place in "de Wolf". It is not only seedplants, forming part of them, to which attention is paid. Also Cryptogams like ferns, clubmosses, mosses, lichens, fungi and Myxomycetes occur in many species and in great numbers.

Among the vegetations there are such as develop without any human interference, while in other cases the human being interferes with the developing vegetation, so with the successionrange, in order to keep alive a certain vegetational type.

The arboretum is that part of "de Wolf" first laid out and planted; in 1930 it was started.

As the arboretum was also meant as a windscreen for the other part of the grounds and it must not obscure the sunlight too much, the greater part of the foliage trees-section is planted on the northside, while the pinetum forms the western border. On the eastside of the grounds a collection of shrubs (fruticetum) is now coming into existence.

About our tree- and shrubcollection as such we will not discourse further here. There are several arboreta in the Netherlands, differing little in principle, though they each have a character of their own.

It is, however, important that in the arboretum of "de Wolf" also the process of vegetation development is allowed to take place under various conditions, so that this arboretum serves a double purpose. For the dendrologist there are the trees and shrubs as such — among which there are some beautiful specimens especially in the pinetum; for the student of vegetations they are the components of the forest, where forestal vegetations develop.

The places where no trees grow, so the open spaces in the forest, are sometimes but small areas, but in other cases they are so large that the trees practically do not influence them. In such places there originate meadows, heaths and swamps.

Methods of cultivation

We have already indicated that, besides the naturally favourable soilconditions to be found at "de Wolf", it is the purposefully organised cultivation of the garden, that helps us to attain the end aimed at, and which also decides the entirely personal character of "de Wolf".

The method of cultivating is quite other than that in "ordinary" botanic gardens and so is quite different from that in the "old" hortus in Groningen also.

Spadework is only sporadically performed, and very locally, only for the benefit of the ruderal plants. The work consists principally of mowing, weeding, cutting, sodding and burning.

When deciding on the methods of cultivation two principally divergent final aims were at issue, roughly speaking.

a. On some grounds we allow a certain vegetation to originate, in this sense that the type, about to develop, has been decided on, e.g. a meadow for plants with a big production of litter, or one for plants with a small production of litter, but here no thought was given to the species which were to be the components of the vegetation.

b. In other areas cultivation is directed towards the development not only of a certain type, but thoughts have also been given as to which plants were to be the components of the vegetation, e.g. a moist heath with *Gentiana pneumonanthe*, *Lycopodium inundatum* etc., a poor meadow rich in orchids, a mossvegetation with *Sphagnum*, *Polytrichum*, *Pogonatum*, *Atrichum* a.o.

There are only two areas where human influence is completely barred. One is a very moist valley, excavated in 1933 down into the boulderclay. Here, via a series of succession stadia, a humid forest came into existence, the composition of which is still continually altering.

The other is a drier tableland, surrounded by a ditch, which was made as late as 1946. On this tableland a rich grassvegetation still dominates now, in 1956; the development into a forest has only just begun. This forest will of course come into existence via quite different successive stadia from that in the valley.

In all other vegetations there is more or less interference by one or more of the methods mentioned above.

This happens only slightly in the remaining forest where only the abundant woody shoots are thinned out. In the meadows which are not mown, only the sparse shoots of woody plants are taken away, while the swamps are saved from turning into swampforests by completely or partially cutting the woody shoots.

The hand of man exercices its influence to a growing extent on the composition of the meadows which are mown resp. once, twice or three times a year. In many of these meadows the moment for mowing is decided on by the vegetation itself; it namely takes place after the most important plants have shed their seeds.

The vegetation is influenced most in those plots for which the combination of species, so the flora, was chosen more or less in advance. Here we also weed, besides mowing or cutting, in places. Sometimes sodding is undertaken; this often occurs on only little trodden paths, and also on the bottom of ditches. Sometimes burning is done, or the substrate is influenced by sanding over. These last measures, just like intensive weeding, are aimed at continually creating new possibilities for the settling of plants with a small, to a very small, production of

litter. Pioneer vegetations or, if the successive series is broken off every year, ephemerous vegetations, are likely to originate here.

Just as was the case when forming the soilrelief, we aim at contrasts in these plots, — so where we interfere intensively — which, exactly as in the remaining part of "de Wolf" are divided into sections, by means of the cultivation-plan, which also makes comparison possible.

This is effected in several ways, viz.:

1. In the greater part of the grounds every section has its own treatment as a unit. Locally however, there often prevail different conditions within such a section. So per section we have to do with a similar method of cultivating, under conditions often greatly varying.
2. In a smaller area every section has its own treatment, while within each section the conditions are entirely or nearly the same.
3. There are a few sections, where, within each section, the conditions show little or no differences. They receive the same method of treatment. Relatively, however, they differ very much in conditions.
4. Somewhere else an area is divided into plots where the same treatment is given under identical conditions, but in each plot at a different time.

It may be advisable to point out that the "cultivationplan" is never deviated from, however great the temptation to do so may sometimes be. It namely happened now and then in the beginning, when competition was not yet great and every plant could settle practically anywhere, that a — let us say "spectacular" plant, e.g. a plant rare in our flora, — settled in an area where we knew it could not maintain itself under the cultivationplan decided on. Yet no other treatment was then undertaken for the benefit of this plant or plants in this special plot.

We may therefore, without any objection, consider human activity at "de Wolf" as a biotic factor.

To the various conditions already mentioned, may be reckoned: macro- and microclimate, proportion of light, moisture status and composition of the substrate. This extremely complicated unity of various methods of cultivation and varying conditions is also directed towards causing a greatest possible differentiation in the process of humification. The course of this process, — and for the pools that of putrefaction — is especially decisive for the development of vegetation at "de Wolf", as, ultimately, also the competition is dependent too on the method and degree of humification.

On: the flora of "de wolf"

Not all the flora of "de Wolf" is spontaneous. As for the woody plants: the trees and shrubs, forming the substance of the arboretum, were planted. A number of herbaceous species, among which also a few not found in our country, were sown or planted. This sowing or planting does not, however, take place anyhow, all over the grounds; certain localities have been destined for it and we use only small quantities and small amounts.

In general we may say that, as for the indigenous plants, all this

concerns species that occur only rarely nowadays, and, as for the exotic ones, species that are characteristic of certain vegetations e.g. alpine rockplants and plants from moist and drier alpine or mountaneous pastures.

When a plant has once been introduced into the garden, we no further interfere with the spreading; this takes place in the same way as for plants occurring quite spontaneously, they themselves "choose" the habitats that suit them best. In certain vegetations the settling of plants is therefore possible, originating from specimens introduced into the grounds in other localities. We call such plants, whether they be trees, shrubs or herbs, "subspontaneous". So their spreading is spontaneous; the term only indicates that the plant was introduced into the grounds by man, in another spot.

Spontaneous are: all Myxomycetes, algae, scalemosses, lichens and fungi; all leafmosses except a few species of *Sphagnum*, most ferns; *Triglochin palustris*, all *Juncaceae*, nearly all *Cyperaceae*, all *Gramineae*, except *Molinia coerulea*, both species of *Typha* (*angustifolia* and *latifolia*), *Potentilla sterilis*, *Achillea ptarmica*, *Cirsium dissectum*. In addition it may be observed that these plants were not introduced into the garden purposely by man; it will, however, always remain possible for them to have come as pollution of imported material.

In 1953 an investigation was made into the occurrence of *Desmidiaceae* in the pools of "de Wolf"; 78 species were determined. Besides these *Desmidiaceae* 23 other algae were determined. To the algae growing on the earth and on treetrunks etc. no attention was as yet given.

Of the scalemosses thus far 21 species were found, of leafmosses 61 species. The number of fungi, determined in the course of years, amounts to nearly 300 species, that of lichens to 36; the number of Myxomycetes is not exactly known, but it certainly amounts to some tens of species.

The remark has been made that it is a pity plants were introduced into "de Wolf" by human being, as "nature" was violated in this way. This point of view is a mistake, as it would not have been possible e.g. to let a heathervegetation originate, if we had not started by planting a few clumps of heather, in other words by forming a centre from where the scattering of seed over the grounds can take place.

Plants like *Gentiana pneumonanthe*, *Lycopodium inundatum* a.o. characteristic in our country of moist heaths, would in all probability never have settled, because they hardly occur in the near neighbourhood of Groningen. It was the intention to form a centre for distribution for all plants we introduced, nothing more.

Another critical remark that is sometimes made, concerns the fact that at "de Wolf" also exotic plants are found, e.g. alpine meadow- and rockplants. It must be kept in mind, however, that "de Wolf" is a botanic garden and that it never was the intention to have only indigenous plants. For the rest the number of these exotic plants, compared to the indigenous ones is very small, and they are only able to maintain themselves in those areas where we interfere inten-

sively. We need not be "afraid" that e.g. an alpine gentian will easily settle in a meadow mown once or even twice a year. Should this occur all the same, let it be so and it is in no wise contradictory to the aim of demonstrating vegetation development, to find out *which* plants can settle and *which* can stay alive for a longer or a shorter period. (This is ultimately the criterion for all vegetation development).

Nowhere at "de Wolf" is there any question about stabilisation, everywhere succession occurs; not only in the vegetation, also the substrate develops and here also one might speak of succession. Substrate and vegetation are everywhere "dynamic", in all sectors.

If it should prove that by the development of substrate and vegetation conditions have nevertheless somewhere become such, as to be favourable to the settling of e.g. an alpine gentian, then this stranger is heartily welcome there. In other words: the main point of work at "de Wolf" lies not on sociological but on ecological (s.s.) territory.

That we introduced a number of indigenous plant-species had yet another reason. They are often species which have become rare in our country, or are becoming so, on account of landreclaiming, ameliorated agricultural methods or for whatever other reason. It was in reality the intention to create a kind of refuge for those plants. And that we met with a certain amount of success may be proved by the fact that nowadays hundreds, if not thousands of plants of *Pinguicula vulgaris* occur, originating from some five specimens, planted in a certain section of the garden. And which scattered quite independantly over the grounds.

The same holds good for *Epipactis palustris*, occurring so overwhelmingly in some places that it assumes a "weed"-like behaviour. *Orchis majalis* and *Orchis maculata* show their manifold types in ample abundance, *Parnassia palustris* has become a "common" plant, *Lycopodium clavatum* covers great spaces.

Less overbearing and at first more or less successful, but now spreading, are: *Orchis morio*, *Orchis militaris*, *Orchis mascula*, *Orchis incarnata*, *Herminium monorchis*, *Platanthera bifolia*, *Listera ovata*, *Gentiana cruciata*, *Gentiana pneumonanthe*, *Narthecium ossifragum*, *Lycopodium inundatum* and *selago*, *Pyrola minor* and *rotundifolia*, *Ophioglossum vulgatum* etc.

It proved that for many of these plants the ecological amplitude is much greater than one would expect. *Pinguicula vulgaris* e.g. settled on peat-dust just as exuberantly as on sand; on loam it grows as easily as on coke-ashes and if there is but a minimal quantity of humus present, it develops as well on calcareous stone as on non-calcareous.

In this way it is made clear by all the plants at "de Wolf" mentioned that they are in no wise tied down to the conditions under which one is apt to find them in the field.

But we can also observe *how* easily most of these plants can be "ousted" and *how* difficult it is for them to conquer new territory. We see with our own eyes at "de Wolf" that only by acting rigorously, by continually causing calamities like the already earlier mentioned

Fig. 1. Herminium monorchis and Epipactis palustris in flower.

Fig . 2. Pool in heath. Part of pinetum on the background.

Fig. 3. View on alpine meadow (left), rocky wall and part of arboretum with foliage trees.

Fig. 4. Rich vegetation of Parnassia palustris.

sodding, burning, sanding over, etc. by which the vegetation extant is entirely or partially destroyed, it is possible to furnish these plants with not only new possibilities for settling, but also new possibilities for expansion. The same holds good, and perhaps even more so for the exotic plants with very small to small fitness for competition, than for the indigenous species mentioned.

And so it happens that in certain localities, after a calamity, beautiful pioneer communities originate, consisting of indigenous plants as well as exotic ones.

By way of example: part of a very damp meadow, bordered by a flowing brooklet, which continually keeps moist not only the meadow but also the lower part of the adjoining rocky wall (i.e. a wall built of sandstones) even in the driest periods.

On the meadows which have been sodded, algae immediately appear, scalemosses and leafmosses establish themselves, a.o. *Polytrichum commune* and bits of *Sphagnum*. Later on we see *Pinguicula vulgaris* appear and *Polygonum viviparum* (alpine), *Tofieldia calyculata* (id.), *Primula farinosa* id.), *Primula luteola* (mountainplant from Central Asia); then *Gentiana pneumonanthe*, *Gentiana cruciata*, *Gentiana asclepiadea* (montane), *Parnassia palustris*, *Arnica montana*, *Lycopodium inundatum* and *selago* appear. *Orchis maculata* and *Epipactis palustris* put forth; *Pyrola minor*, *Drosera rotundifolia*, *Vaccinium vitis-idaea*, *Calluna vulgaris* and *Erica tetralix* settle. (We must immediately take measures against these last three species, as the meadow would otherwise become a „heath" within a few years).

It is more or less astonishing when it becomes apparent that a number of those plants has also settled in a place where one would not expect them, viz. in the crevices of the adjoining stonewall. It was not at all our "intention" that *Parnassia*, *Drosera*, *Gentiana* and *Lycopodium inundatum* would settle there.

The wall was intended for alpine rosette plants, on the drier parts *Sempervivum*, *Androsace* etc., in the more humid spots species of *Saxifraga*. These Saxifrages have found favourable conditions for their growth after lengthy wanderings. From the spots where a few rosettes were planted, they vanished long ago; there *Sempervivum* settled, which in its turn now fights a "struggle for existence" against oncoming lichens and mosses.

Amazing though this settling of *Parnassia*, *Drosera* etc. against the wall may have been, it can be understood. Seeds had been produced in abundance by the specimens along the brook; there was hardly any competition in the crevices between the stones; there was but little development of *algae*, just not too strong to prevent germination; the conditions of humidity and light apparently were exceptionally favourable and so we find besides the plants mentioned also a young plant of *Rhododendron hirsutum* (seed from the *Rhododendron* valley), seedlings of *Calluna* and *Erica* and even young plants of *Osmunda regalis* (spores from moist forest) and a few more species of ferns.

On the piece of sodded meadow described above we had occasion to ascertain that the spores and prothalliums of *Lycopodium inundatum*

indeed only need a short period for development. Already in the third summer after the sodding several plants were present.

It will hardly be necessary to remark that the development on other vegetation-free plots for which the conditions differ, will be entirely different.

We do not consider it necessary to treat all this in extenso. Once again: let us point out emphatically that all those processes enact on a relatively small part of "de Wolf", and that in the greater part of the garden human interference remains restricted to more or less intensive mowing.

Herewith we conclude this summary of what goes on at "de Wolf" and what biological processes are enacted.

One more final observation. At the beginning of this paper we simply stated: Aim II is the creating of possibilities for growth for as many plants as possible, Phanerogams as well as Cryptogams. However: what a fascinating idea; what possibilities lie hidden there.

To be able to give an idea, within a limited space, of the exceptionally complicated processes of humification under various conditions and the effect of it all on the vegetation; to be able to give an idea of succession; to put into practice the possibilities to guide this succession, to direct it towards an aim decided on in advance etc. etc.

One must realise what this infers, how many questions again and again come thronging, what hoard of observation may be gathered; observation, not only of botanical importance but possibly also essential in the practice of conservancy and eventually the establishment of nature-territories.

SUMMARY OF THE PRINCIPAL VEGETATIONS OCCURRING IN THE GROUNDS OF "DE WOLF"

A. Woodvegetations

1. Foliage trees, Conifers. Not mown. Herbs with great production of litter; hardly any mosses or fungi.
2. Foliage trees, Conifers, promiscuous forest. Mown once a year. Herbvegetation with less production of litter. In the last section rather more mosses and fungi than in the former two.
3. Foliage trees, Conifers, promiscuous forest. Mown twice a year. Herbs with little production of litter, mostly springflowering. In the promiscuous forest e.g. many *Anemone nemorosa, Ranunculus ficaria* and *auricomus*. Into the pinetum heath penetrates, especially in the lower parts and the open spaces.
4. Through the entire wooded area broad strips, also meant for paths, mown several times a year. Hardly any herbvegetation; abundant development of mosses and fungi.
5. In the entire wooded area spontaneous shoots of foliage trees. In certain sections no interference by man; here a "jungle" originates. In other places this development is checked by cutting.
6. In several places in the forest, where socalled trunk- and branch-manuring is practiced, abundant development of woodfungi, mosses and Myxomycetes, while gradually ferns settle on the decayed wood.

B. Dams

Partly unplanted, partly planted with foliage trees and with Conifers. Mown or not; in the former case mosses, lichens, fungi but little herbgrowth; in the latter rich development of herbs.

C. Ruderal vegetations

1. Wide area in deciduous forest, where every year the wood — to be thinned out — of small proportions is burned. Here also plants characteristic of such habitats, such as the moss *Funaria hygrometrica* and the fungus *Flammula carbonaria*.
2. Sections outside the forest, dug up in spring and autumn. "Weeds".

D. Meadowvegetations

There are meadows within the forest and without. In the first case the vegetation is influenced by leaf- or needle-litter and by other "forestal conditions".

1. Foliage-tree meadows and needletree meadows. Not mown. Herbvegetation with very great to great production of litter. No mosses or fungi.
2. Id. Mown once a year. Herbgrowth with less production of litter. Little development of mosses and fungi, in needletree meadows more than in foliage-tree meadows.
3. Id. Mown several times a year. Little herbvegetation. On the other hand many mosses and fungi.
4. Meadows outside the wood, so where the above mentioned influences of forestal conditions do not exist.
   a. Not mown. Herbvegetations with great litter production. Mosses nor fungi.
   b. Mown once a year. Herbvegetations with less production of litter. Mosses and fungi.
   c. Cut and weeded, so very intensive human influence. Herbs with very small production of litter. We also interfere in the mossvegetation; development of *Sphagnum* however is stimulated. After years the *Sphagnum* vegetation probably will become so dense that it starts to dominate; then interference will be necessary there too.

   One of these meadows might be described a little more in detail, viz. the orchid meadow.

After the moment at which the most important plants have shed their seeds, the meadow is mown and the refuse is immediately removed. The impovering gradually progresses and the meadow, in which grasses dominated, begins to adopt the character of the socalled "bluegrass meadow". *Carices* penetrate and a plant like *Cirsium dissectum* which is typical in such meadows, settles there quite spontaneously.

Early in spring plants like *Anemone nemorosa, Fritillaria meleagris, Primula elatior,* flower here. Somewhat later there appear *Lychnis flos-cuculi, Pedicularis palustris, Ajuga reptans, Rhinanthus glaber, Filipendula ulmaria, Molinea coerulea* and orchids: *Orchis majalis, Orchis maculata, Orchis morio, Orchis palustris, Listera ovata, Platanthera bifolia* and *Gymnadenia conopsea*. But also mosses like *Climacium dendroides* and fungi like *Sclerotinia tuberosa* and species of *Hygrophorus* occur.

Besides the above mentioned meadows there are at "de Wolf" also a number of meadowtypes in the making, where we will try to change only one factor at a time.

1. There are four meadows side by side under the same conditions of light and humidity, which also receive the same method of cultivation (mown a few times a year) on 4 greatly differing substrates, viz. a. on loamy sand, rich in humus; b. on calcareous seasand; c. on lime marl; d. on coalashes. The differences in overgrowth were extremely remarkable already in the first year; the further development can be studied.
2. Three meadows develop on loamy sand, rich in humus, and under the same conditions of light and cultivation (mown a few times a year) but under different conditions of humidity; a. moist; b. drier; c. dry.

For six meadows, on loamy soil, rich in humus and under similar conditions of light and moisture, the identical cultivation is practiced (mown once a year) viz. respectively on the 1st day of the months June to November inclusive.

E. Heaths

The grass- and herbvegetation is either mown here, or cut or weeded. If necessary the development of mosses too is checked, that of *Ericaceae* and *Sphagnum* on the

contrary is stimulated. It is not the intention ever to interfere with the growth of *Sphagnum* in heaths.

1. *Myrica*- and *Vaccinium* heath. In a shallow dell, where water stagnates in a few places in winter only. As yet no *Sphagnum* development.
2. *Calluna*- and *Erica* heath. There are two large and a series of small dells in it. A great part of this heath is submersed all winter long.

   This heath, where *Empetrum nigrum* has been introduced, harbours plants we might expect on such soil in nature just as well; *Pedicularis sylvatica*, *Gentiana pneumonanthe*, *Arnica montana*, *Orchis maculata*, *Sphagnum* cushions; in deeper pits *Narthecium ossifragum*, *Eriophorum angustifolium*, or on a peaty soil *Andromeda polifolia* and *Oxycoccus quadripetalus*; in more bare places (sodded patches, mostly on paths) *Drosera rotundifolia*, *Pinguicula vulgaris* and *Lycopodium inundatum*.
3. *Pinus montana*- and *Rhododendron* heath. Principally alpine species.

F. Walls and stony meadows

Here human interference is very great on account of repeated weeding. Only herbs with a very slight production of litter, such as alpine herbs and rosetteplants are tolerated. On the stones many mosses and lichens.

G. Pools

As has been made clear in the part treating the water-provision, there are brooks and pools with eutrophic subsoil water and others with oligotrophic rainwater. The former type of water on the one side feeds a few small pools in the promiscuous forest and flows past mown pastures, where it makes it possible for *Sphagnum* to develop, while on the other side it flows through foliage tree-forest and part of the meadows where it provides water for a pool which is choking up, and for a few other pools of various depths. In, and bordering, these pools an abundant growth of water-, swamp- and shoreplants.

The oligotrophic rainwater is caught in the pinetum and on the heaths (vide eo loco) in more or less deep valleys without drainage. They have all been excavated in the boulderclay-soil so that no water is lost by percolation. Where the water stagnates very much, *Spaghnum* cushions develop, which have been evidently growing these last few years.

In the pinetum we sometimes tolerate leaftree-shoots in the dells, so that a swamp-forest may originate; in other places it is destroyed by cutting.

In the *Pinas montana-Rhododendron*-valley the rainwater stagnates in a small runnel. Along the sides development of *Spaghnum*.

Further there are four pits on the southborder of the grounds of various size and depth, excavated in the clay and bordered by claydams. The first, shallow, is without water for some time in dry summers; tree- and herbshoots are then taken away. This oligotrophic pool has a.o. already many remarkable micro-organisms.

On the dams of the second, deeper, pit, which never quite falls dry, a strong development of *Polytrichum commune* originated on the bare clay.

Of late we see it die down, while new growths do not appear. Along the shores of the pool much *Marchantia polymorpha*, *Pellia epiphylla*; in the pool algae, a.o. *Chara vulgaris*.

The 3rd and 4th pits are not bordered by dams; they are part of a valley which is submersed in winter.

Here a.o. magnificent growth of *Hypericum humifusum*.

The valleys and pits in this area are bordered by rows of Conifers and of leaftrees resp.

Further there are still a few other dells, lying in the leaftreeforest and in the meadows. Some have stagnating water in winter, others have not. There is, however, in all those valleys sufficient water to enable the growth of resp. a hygromorphous forest- and a hygromorphous meadow-vegetation.

Of course it is impossible, in this short survey, to describe everything that happens in the botanic garden "de Wolf", to discuss all the plants that occur there. A more explicit description of only a few sections was chosen, while the photo's reproduced here also give an impression of the grounds.

There is continuous vegetation in nearly all sections of the garden and most

plants have no fixed place, chosen by the gardeners, but they themselves "choose" there spot for development. The chance that they find habitats congenial to their growth is great because there are so many different conditions in "de Wolf".

We have tried to give an impression of the exceptional character of "de Wolf" in this paper and also of the great variety of vegetationtypes which have developed there in the course of about 25 years.

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THE ACTIVITY OF STARCH-HYDROLYSING ENZYMES IN PEARS DURING DEVELOPMENT AND COLD-STORAGE

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Introduction

The keeping qualities of fresh vegetables and fruit can be prolonged by placing them in cold-storage. This counteracts the development of micro-organisms on one hand and slows down the life processes on the other. As the various physiological processes do not as a rule react identically to a reduction in temperature, disharmony may be created in the living object. This does not often prove to be a serious difficulty in the case of products having reached the final stage of their development prior to entering cold-storage, as long as the ensuing transformations are limited. This disharmony, however, is often the cause of storage being restricted to a relatively short period.

There is a further complication in the case of apples and pears, however, in that the fruit must complete its entire ripening after the period spent in cold storage before it is fit for consumption. This ripening is generally considered to be the functional breakdown of the tissue. Should excessive disharmony be created in the fruit during cold-storage, normal ripening is rendered impossible and the fruit can never become fit for consumption.

The behaviour of pears in cold-storage is partially governed by their stage of development, in other words by the activity of the enzyme systems at the moment the fruit was gathered.

The pears are invariably picked in an unripe condition; the subsequent ripening process being of longer or shorter duration, depending on the variety. Fruit picked too late actually ripen during cold-storage, whereas fruit picked too early will not ripen after leaving cold-storage. The interval of time between too early and too late picking is fairly short. This is not the case, however, with pears intended for direct consumption; the dates on which these pears can be picked may vary considerably, e.g. be spread over a period of 3-4 weeks.

We set out to study the activity of α- and β-amylase during the development of pears. The investigation also included the changes in

enzymic activity during the cold-storage of the fruit at 0.5° C. In addition, we endeavoured to ascertain the influence of the time of gathering on these changes and whether a number of varieties exhibited characteristic differences in this respect. Our inquiries also extended to the good ripening potentialities of each group after various periods in cold storage. The principal reason underlying this investigation was the phenomenon that starch is formed in pears during the course of their development, only to disappear again subsequently (see Fig. 4a). The first small amounts of starch can be found in the fruit, close under the peel, towards the end of June. The quantity of starch increases rapidly, spreading throughout the fruit and reaching its maximum about the beginning of August. From then onwards the quantity of starch decreases rapidly; towards the middle of September but little remains. As long as the pears are still on the tree, a small amount of starch is present in the fruit. This is generally the case, too, when normal ripening begins. As soon as the fruit has been gathered, the starch is broken down fairly quickly, disappearing completely within three days to a week. This degradation also continues, albeit more slowly, during cold-storage, so that no starch is present in cold-storage pears when ripening begins.

Even though numerous investigations have been made of the changes taking place both during the development as well as the cold-storage of fruit [Biale (1950), Nitsch (1953), Smock (1944), Smock and Neubert (1950)], only a few research workers have paid attention to enzymic activities up to the present [Ezell and Gerhardt (1938, 1942), Weurman (1954a and b)].

Materials and methods

The pears used for the amylase determinations were mainly of the Doyenné Boussoch variety. Economically these pears are not of great importance as their flavour qualities are not rated highly This variety was selected, however, because it ripens well after cold-storage until March.

These pears were available in adequate quantities from one orchard, so that we were assured of a constant supply of pears of identical origin during a given growth and storage period, a factor of great importance when making series determinations. The orchard from which the Doyenné Boussoch pears were obtained is stocked with trees approximately 40 years old, so that they can supply a considerable quantity of fruit in good years. The orchard is at Langbroek near Doorn (Province of Utrecht). Soil type: heavy clay. Each sample consisted of 15 pears (more at the beginning of the season).

As the changes in amylase activity were characteristic, it appeared interesting to extend these investigations to two other varieties of pear, differing from the Doyenné Boussoch in the matter of their storage potentialities. Our choice fell on Conférence, one of our best dessert pears, and Comtesse de Paris; on the former because Conférence pears generally lend themselves well to storage over a lengthy period while preserving their ripening potentialities; on the Comtesse de

Paris, because difficulties frequently arise in connection with its ripening after cold-storage.

Both these varieties were obtained from a model orchard at Hoofddorp. Soil type: good sandy clay. The trees were much younger, viz. 15 years old. We used a row of 20 trees of identical age for the series determinations in connection with each variety. A sample consisted of 20 pears, one taken from each tree.

Activity determinations in individual pears showed that individual differences in amylase activity were so great that deviations up to 30 % in samples of 15-20 pears cannot be regarded as essential (MARIS MCARTHUR, 1955).

During the collection of these samples, care was taken to avoid gathering particularly small or large individuals. The pears were washed and dried; proportional parts by weight were taken by cutting a longitudinal section from each pear. These segments were then peeled, the core removed, rasped and mixed together.

The quantities of starch and the activity of the α- and β-amylase, inter alia, in the samples were determined. The α- and β-amylase activity was expressed as the number mg starch broken down by the α- and β-amylase respectively per 100 g pear pulp in one hour at 25° C. This activity is frequently expressed per pear in the following pages.

The starch content was determined by the method of LOOMIS and SHULL (1937). A few corrections had to be made for the material under examination (MARIS MCARTHUR, 1955).

For the determination of the α- and β-amylase we empolyed a method whereby the colour changes in starch-iodine and starch-erythrodextrin complexes respectively were measured. This method was developed by HOSKAM (1947) for determining amylase activity in various flour types. We have used this method successfully on pear material.

Erythrodextrin, which is broken down by α-amylase and not by β-amylase, was used as substrate in the determination of the α-amylase activity. Erythrodextrin is coloured red by iodine; this colorability disappears during hydrolysis by α-amylase. The activity of the α-amylase can be determined from this change, which can be followed colorimetrically. The activity of β-amylase cannot be measured separately as no substrate is known that is preferentially hydrolysed by β- and not by α-amylase. The combined activity of α- and β-amylase is therefore determined, soluble starch being added as substrate. Here too the colour change is measured colorimetrically. By subtracting the value obtained for the α-amylase alone from that for the α- and β-amylase together, the corresponding figure for the β-amylase can be calculated. As starch and erythrodextrin are not broken down at the same rate by α-amylase, it was necessary to ascertain the ratio between these rates under the test conditions. We found a value of 0.76 for this ratio, which also corresponds with that found by HOSKAM. For both determinations we employed a filter with a maximum transmission of about 572 mμ.

Further details of determinations in pear pulp are described elsewhere (MARIS MCARTHUR, 1955).

Tests of the amylase determinations in pears

HOSKAM's method only yields reliable results providing a number of conditions are satisfied:

Activity determinations must be made at the optimum pH. The pH of the reaction mixture at which pear amylases evidence optimum activity was therefore investigated. Fig. 1 shows that the pH range between 5.7 and 6 is most favourable for the activity of $(\alpha + \beta)$ amylase. The optimum breakdown of erythrodextrin by α-amylase occurs at a pH of about 6. All our amylase determinations in pear pulp were therefore carried out at a pH between 5.7 and 6.

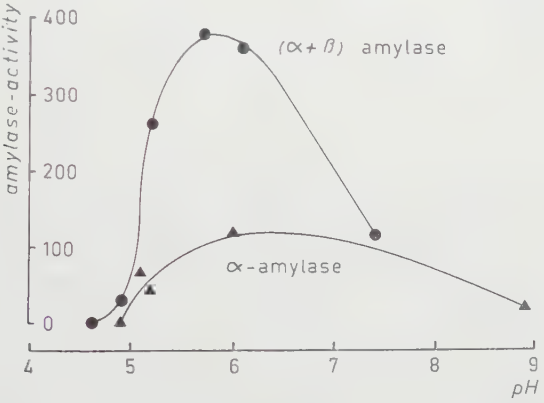

Fig. 1. *The influence of pH on the activity of* $(\alpha + \beta)$ *amylase (substrate: starch) and* α*-amylase (substrate: erythrodextrin) in pear pulp.*
Coarsely rasped pulp macerated with $NaHCO_3$; pH of pulp 6.2 — Samples modified to various pH with 0.5 N HCl or 5 % $NaHCO_3$ and made up to 15 ml with water — Reaction mixtures: 10 g pulp, 10 ml water and 20 ml starch solution 1.25 % or erythrodextrin solution 1.25 % — Duration of the reactions: 30-70 minutes — Temp. 25° C.

The determinations must be made in the presence of an excess of starch (or erythrodextrin) in order to ensure that the starch in pears does not influence the results. Our tests showed that the rate of starch breakdown increases as the concentration is raised, but that hydrolysis remains independent of the concentration for several hours in a reaction mixture containing 0.5 % starch. In this case the enzyme is saturated with substrate (see Table 1). All amylase determinations were, of course, executed in such a way as to ensure that this enzyme saturation was invariably achieved. This was certainly the case when the rate of hydrolysis, in a reaction mixture containing 0.6 % starch, did not exceed 0.8 mg per minute.

α-Amylase was saturated with erythrodextrin in a concentration of $\pm$ 0.4 % erythrodextrin (see Table 2).

For these determinations we used starch and erythrodextrin concentrations of 0.6 %, ensuring by dilution of the pulp that the reaction rate was lower than 0.5 mg per minute.

The calculation of the enzymic activity can be greatly simplified

TABLE 1

Influence of the starch concentration on the rate of hydrolysis by ($\alpha + \beta$) amylase in pear pulp (high starch concentration)

Doyenné Boussoch pears — buffered starch solution 2.5 %, pH 5.6 — Reaction time: 100 minutes — Temp. 25° C — Constantly stirred

Pear pulp	10 g	10 g	10 g	10 g
Water	30 ml	25 ml	20 ml	15 ml
Starch solution	10 ml	15 ml	20 ml	25 ml
pH reaction mixture	5.82	5.79	5.75	5.71
Concentration starch in reaction mixture	0.50 %	0.75 %	1.0 %	1.25 %
Starch hydrolysed per minute	0.53 mg	0.56 mg	0.52 mg	0.52 mg
Activity ($\alpha + \beta$) amylase	320	335	310	310

TABLE 2

Influence of the erythrodextrin concentration on the rate of hydrolysis by α-amylase in pear pulp

Doyenné Boussoch pears — buffered erythrodextrin solution 0.625 %, pH 5.6 — Reaction time: 90-120 minutes — Temp. 25° C — Constantly stirred — Determinations of α-amylase activity.

Pear pulp	10 g	10 g	10 g	10 g
Water	30 ml	20 ml	10 ml	—
Erythrodextrin solution	10 ml	20 ml	30 ml	40 ml
pH reaction mixture	5.87	5.80	5.80	5.80
Concentration erythrodextrin in reaction mixture	0.13 %	0.25 %	0.38 %	0.50 %
Erythrodextrin hydrolysed per minute	0.25 mg	0.35 mg	0.48 mg	0.44 mg
Activity α-amylase	—	—	220	200

by selecting the test conditions so that the rate of hydrolysis is proportionate to the time. It proved possible to realize this by permitting the enzymic process to take place at the optimum pH, adding adequate substrate to saturate the enzyme, maintaining the temperature constantly at, say, 25° C, and stirring the reaction mixture constantly and intensively. If the latter is neglected, the suspension precipitates rapidly, resulting in part of the enzyme present becoming less active.

It appeared that the rate of hydrolysis remains constant under the conditions referred to in the foregoing. This will be seen from the graphs at Fig. 2.

The rate of hydrolysis only decreases when the reaction has progressed to such an extent that there is no longer any excess substrate present. As this rate remains constant, even when the test is of longer duration, this value can be determined with sufficient accuracy.

In order to be able to calculate the enzymic activity in pear pulp, we had to ascertain whether the rate of hydrolysis was proportionate to the quantity of pulp. This is shown at Fig. 3.

Results of the amylase determinations in different varieties of pears

α- and β-amylase determinations were made at regular intervals in several varieties of pears, during the growing season, the period in

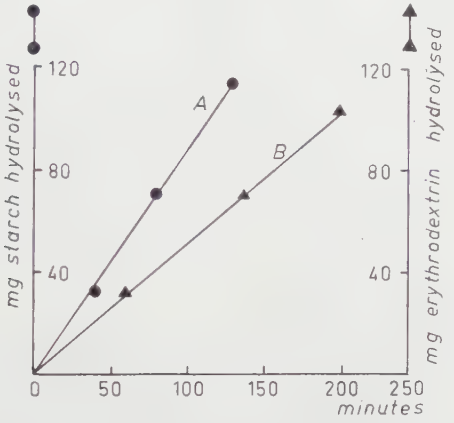

Fig. 2. *Hydrolysis under optimum conditions.*

A. *Starch as substrate.*
Doyenné Boussoch pears — Reaction mixture: 30 g pear pulp, 30 ml water and 60 ml starch solution 0.625 % — pH 5.62 — Temp. 25° C — Constantly stirred.

B. *Erythrodextrin as substrate.*
Doyenné Boussoch pears — Reaction mixture: 15 g pear pulp, 15 ml water and 30 ml erythrodextrin solution 0.625 % — pH 5.7 — Temp. 25° C — Constantly stirred.

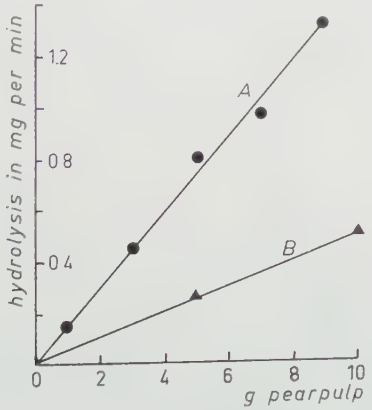

Fig. 3. *Influence of the quantity of pear pulp (enzyme) on the rate of hydrolysis.*

A. *Starch as substrate.*
Doyenné Boussoch pears — Buffered starch solution 1.25 %, pH 5.8 — Reaction time: 1-2 hours — Temp. 25° C — Constantly stirred.

B. *Erythrodextrin as substrate.*
Doyenné Boussoch pears — Buffered erythrodextrin solution 0.625 %, pH 5.8 — Reaction time: 2 hours — Temp. 25° C — Permanently stirred.

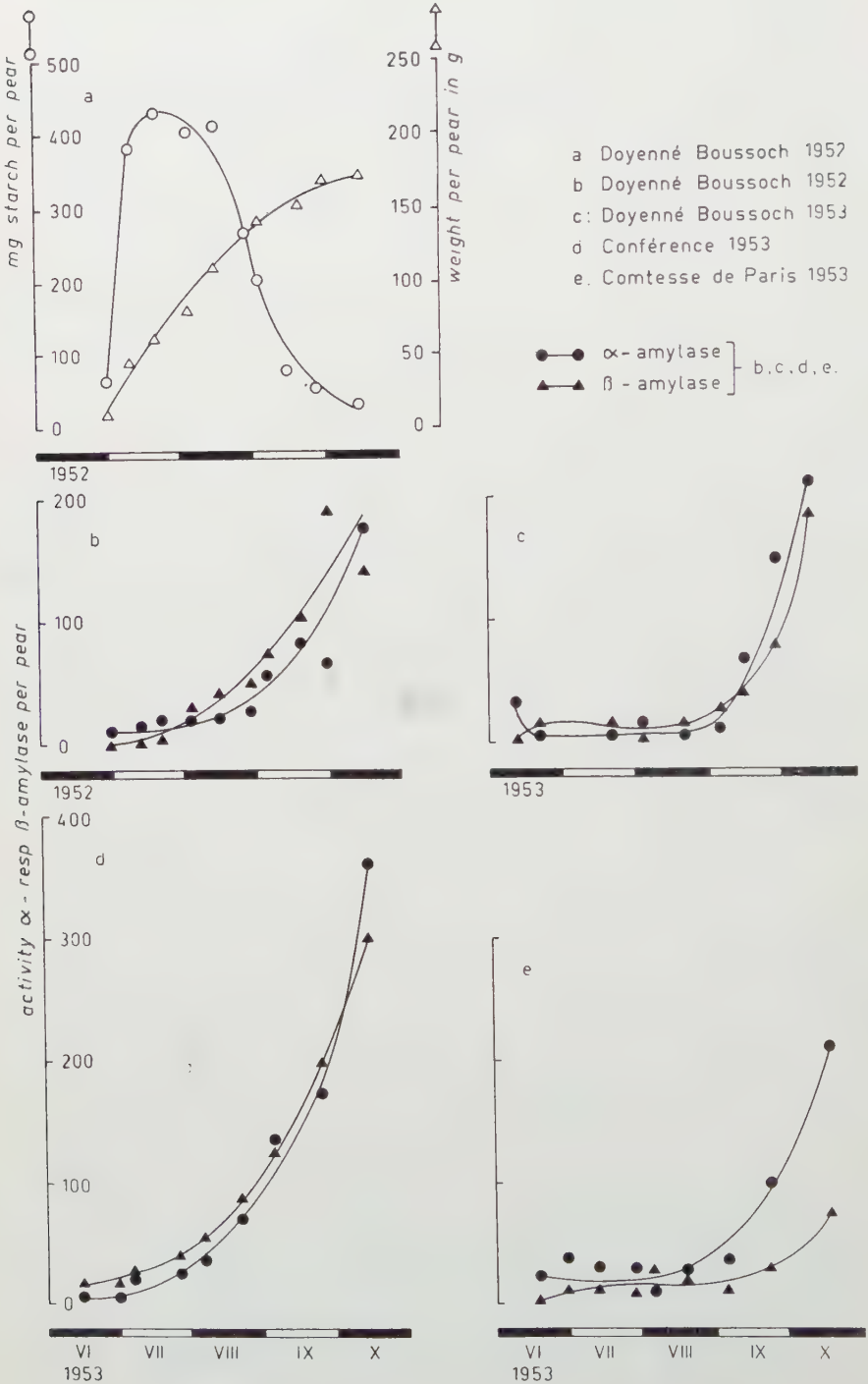

Fig. 4.

a. Average weights and quantities of starch per pear of Doyenné Boussoch in 1952.
b-e. Quantities of α- and β-amylase per pear during development on the tree.

cold-storage and ripening. In addition, samples of cold-storage pears gathered on different dates were also compared with one another.

The growth period

The amounts of amylase present during growth on the tree are summarized at Fig. 4. In each case the amount was calculated separately per pear. In addition, the average weights of the pears and the quantities of starch per pear are shown at Fig. 4a. The starch content reaches its maximum towards the end of July.

The general impression is that the amounts of both α- and β-amylase increase only slightly during the first half of the growth period, but fairly sharply during the second half. In general, the trends are identical for all three varieties, differing only in minor details.

A striking fact is that the major increase in the amount of amylase begins after the largest quantity of starch has already disappeared.

The storage period

Fig. 5a shows the amounts of α-amylase per pear in the three varieties during cold-storage. The earliest gathering dates were selected in this case (for more detailed figures regarding all gathering dates, see Maris McArthur, 1955).

The graph shows clearly the period up to which ripening is possible.

It will be noted that the increase in the amounts of α-amylase, which actually began on the tree (see Fig. 4) still continues during cold-storage to such an extent, that the quantities of α-amylase achieve values never reached on the tree.

The increase in the amount of α-amylase presents an entirely different picture in each of the three varieties. The increase is only slight in the case of Conférence pears, enormous in Comtesse de Paris, while it lies between the two in the Doyenné Boussoch variety.

Fig. 5b shows the amounts of β-amylase during the storage period. It appears that no important changes in the quantities of β-amylase occur in any of the three varieties.

The ripening

Our investigations also extended to ascertaining whether any changes in amylase activity took place during ripening. To determine this, cold-storage pears of the Doyenné Boussoch variety were stored for some time at room temperature during various stages of the investigation. The results are incorporated in Table 3.

This table shows that changes do actually take place during ripening. There is an obvious decrease in the amount of α-amylase, the differences being much greater than the anomalies which could possibly be attributed to the sampling methods; the amount of β-amylase varies irregularly; it is possible that it undergoes practically no change.

Meanwhile, two other control determinations had to be made before it could be concluded with certainty that there is a marked biosynthesis of α-amylase during the cold-storage of pears. After all,

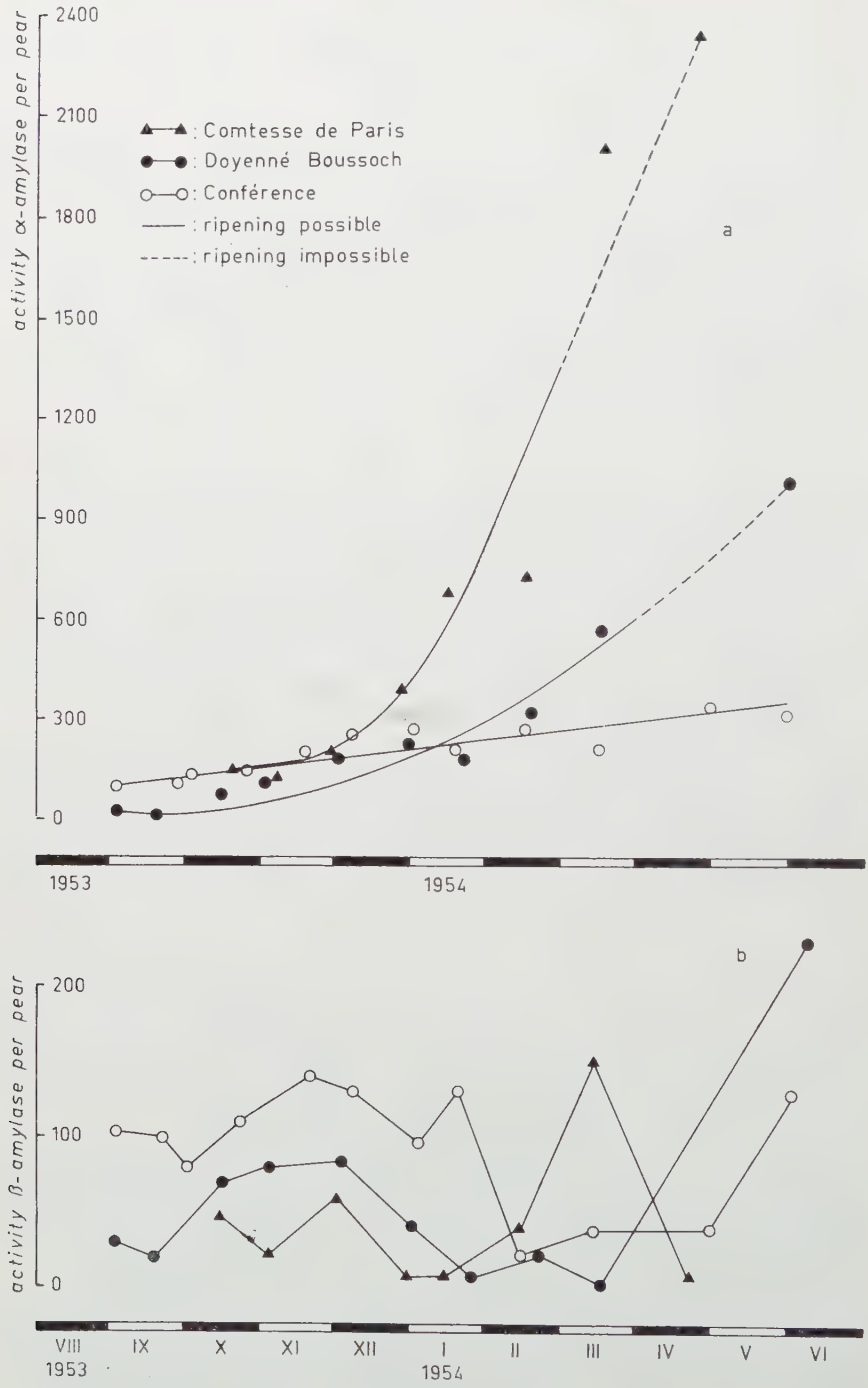

Fig. 5.
a. Quantities of α-amylase per pear during cold-storage.
b Quantities of β-amylase per pear during cold-storage.

TABLE 3

Changes in α- and β-amylase activity during the ripening of Doyenné Boussoch pears

Pears gathered unripe on 10/9/52 — Kept in cold-storage at 0.5° C — Ripening at 23° C

	Date test	Details	Activity α-amylase	Activity β-amylase
A.	8/1/53	Unripe pears from cold-storage ...	90	55
	16/1/53	9 days ripening, ripe for consumption.	55	35
B.	23/1/53	Unripe pears from cold-storage	115	55
	28/1/53	7 days ripening, ripe for consumption.	85	90
	3/2/53	12 days ripening overripe and mealy .	35	70
C.	11/2/53	Unripe pears from cold-storage	245	95
	17/2/53	7 days ripening, ripe for consumption.	180	75
	25/2/53	15 days ripening, over-ripe and mealy .	45	80

it is feasible that the observed changes in activity could be attributed either to the presence of amylase-inhibiting substances during the evolution of the pears, or to the formation of amylase-stimulating compounds during the cold-storage of the fruit. The following experiments were conducted in an endeavour to elucidate this question.

Tests were made at various times to ascertain whether macerated pear pulp exerted any influence on the activity of α- and β-amylase preparations, prepared from malt and pearl barley respectively. This involved determining the amylase activity of the macerated pear pulp and that of the amylase preparation on one hand, and that of both together on the other. The results of a few of these experiments are presented in Table 4.

This table shows that between August and January pear pulp exercised a slight inhibitive action on the activity of the α- and β-amylase preparations. This inhibition, however, amounts at the most to one-third of the total activity. If inhibiting substances are actually present, then their action must be so weak that it can have no practical significance in explaining the observed changes in the activity of the amylases.

It follows from these observations too that no substances occur in the pulp of pears kept in cold-storage, which stimulate the amylase activity. If this was the case, the breakdown of the starch by an amylase preparation plus pear pulp would have been greater than the sum of the breakdown by both components individually. This was not the case however.

Consideration also had to be given to the possibility of combined amylases occurring in pears. It is known that α-amylase occurs in

TABLE 4

Influence of macerated pear pulp on the activity of α- and β-amylase preparations

Doyenné Boussoch pears, finely ground with $NaHCO_3$, pH pulp 5.7 — α- and β-amylase solutions in 0.2 M acetic acid-acetate buffer, pH 5.7. Buffered starch solution of 1.25 %, pH 5.7 — Reaction mixtures consisting of: 10 ml enzyme solution/10 g pear pulp, 10 ml water and 20 ml starch solution — or — 10 ml enzyme solution, 10 g pear pulp and 20 ml starch solution.

Date	Enzyme preparation	Starch breakdown in mg/hour		
		Amylase	pulp	Amylase + pulp
12/ 8/53	β-amylase	18	3	13
13/ 8/53	α-amylase	24	1	19
1/ 9/53	α-amylsae	26	4	20
6/10/53	α-amylase	29	1	22
26/11/53	β-amylase	60	16	70
27/11/53	α-amylase	34	24	46
19/ 1/54	α-amylase	20	0	12
20/ 1/54	β-amylase	52	5	51
26/ 4/54	α-amylase	26	44	71
27/ 4/54	β-amylase	36	21	58

combined form with protein in barley grain; the activity of this enzyme, however, is then weak. This linkage can be broken by the addition of a proteolytic enzyme — papain — resulting in the α-amylase activity increasing considerably, as has been demonstrated by experiments of Ford and Guthrie (1908).

β-amylase, too, can be liberated in this way from oat grain.

This increase in the amylase activity of germinating seed such as barley, is probably due to the action of a protease.

If the amylases occurred in combination (probably) with proteins in the pears, it would be possible for the increase in activity, which we found during the cold-storage of pears, to be ascribed not to a change in the amount of amylase, but to the breakdown of this inactive complex. To establish this, an investigation was made to ascertain whether there was any increase in the activity of the amylases consequent upon the interaction of papain. Doyenné Boussoch pears, kept in cold-storage to the end of January or the beginning of February, were selected as test material. The greatest increase in activity took place thereafter, so that it could be assumed that — if this hypothesis was correct — the interaction of papain would have the greatest effect.

A number of experiments were conducted, during which papain — to which cysteine had been added as an activator of the papain — was allowed to interact on macerated pulp at pH 7.5-8, a little toluol also being added. The pH of the reaction mixture was subsequently modified to 5.7 and the amylase activity determined.

It appeared that there was absolutely no question of any increased amylase activity consequent upon the interaction of papain.

The results of these experiments may be summarized by stating that the increase in α- and β-amylase activity during the last stage of

growth of pears and the increased α-amylase activity during cold-storage can only be explained by a powerful biosynthesis of both enzymes.

Discussion of the results

The extremely intensive biosynthesis of α-amylase in cold-storage pears is a remarkable phenomenon, especially when it is remembered that no substrate whatsoever for the enzyme is present in cold-storage pears. This becomes even more interesting when we compare these observations with the ripening potentialities of the pears (see Fig. 5a): Conférence, the variety exhibiting only a slight increase, continued ripening until the bitter end (stocks were exhausted by the end of June). Comtesse de Paris, in which the increase was very marked, continued ripening until the end of February. Doyenné Boussoch, intermediate between the two other varieties in this respect, continues ripening until April.

There is thus a parallel between the amount of α-amylase at a given moment and the good ripening potentialities. This potentiality holds good for pears providing the increase in the amount of α-amylase is not excessive. If it is excessive, on the other hand, the possibility of ripening disappears.

The parallel between the increase in α-amylase and the ripening potentialities is also apparent from a comparison of the first and second gatherings of Comtesse de Paris. The pears of the first gathering revealed a more rapid increase in the amount of α-amylase than those of the second. Corresponding with these facts the pears of the first gathering were able to ripen for a shorter period (up to the end of February) than those of the second (up to the end of April).

If we regard an abnormal increase in the amount of α-amylase as a symptom of a disturbed harmony in the cold-storage fruit, it is quite feasible that these disturbances might well reach such proportions at a given moment that ripening becomes impossible.

An approximate calculation (see Maris McArthur, 1955) shows that the following holds good for the Doyenné Boussoch and Comtesse de Paris varieties: if the α-amylase activity, expressed per 100 g pulp (A_a), reaches a value 6-8 times higher than what it was about the time the fruit was gathered, ripening is no longer possible. The A_a in respect of the Conférence pears never reached this value; these pears can therefore ripen well for a very long time (until June).

We thus have an indication as to whether cold-storage pears can subsequently ripen well or not in the ratio between the value for the activity of the α-amylase (A_a) in these pears at a given moment and that for A_a around about September and October. Each variety possibly has its own critical ratio value.

The fact that there is a correlation between the changes in the A_a and the ripening potentialities does not imply that there must be a direct causative link between the two phenomena. The modified α-amylase activity should be regarded more as an indication of a disturbance in the harmonic equilibrium.

These investigations show that not all processes are necessarily delayed by cooling. The influence of a low temperature may be such that a process can continue at the same rate or even accelerate. This applies to the biosynthesis of α-amylase in pears. Certain materials may consequently be accumulated. In the case under investigation this was due to an enzyme unable to find a substrate. This could well be the case, too with enzymes that actually find a substrate; the result might be that conversions occur that would not take place under natural conditions, and if they did, to a far lesser extent.

The changed composition of the fruit — especially as far as the enzymes are concerned — may lead to such disharmony that normal ripening is no longer possible.

The accumulation of amylase is a phenomenon which probably has no repercussions for the fruit. Quite accidentally, the α-amylase activity curve proved to be an indication for the ripening potentialities at a given moment.

SUMMARY

Some varieties of pears can no longer ripen after spending some time in cold-storage.

An investigation has been made of the changes in the activity of a small number of enzymes in this fruit during growth and cold storage. The changes in the activity of α- and β-amylase in three varieties of pears, gathered at different dates, were examined; these varieties differ considerably from the point of view of their post cold-storage ripening potentialities.

During the pear's development on the tree, the quantities of α- and β-amylase increase to a certain extent. During cold-storage, the quantity of α-amylase continues to increase, reaching a value which is never achieved under natural conditions. The rise in α-amylase activity during cold storage proved to be entirely different for the three varieties of pears.

There is a certain parallel between the rise in α-amylase activity and the possibility of ripening after cold storage, which was also found when a comparison was made of the first and second picking of the variety Comtesse de Paris. If the increase is not too excessive, ripening remains possible. If the rise in α-amylase activity is too great, at a rough estimate six to eight times the value at commercial picking-time, ripening is rendered impossible.

In the ratio between the value for α-amylase activity in pears during cold-storage at a given moment and that about commercial picking time, we found an indication as to whether pears can still ripen or not. It is possible that a specific critical value exists for each variety.

ACKNOWLEDGEMENT

I am very much indebted to Professor Dr. A. W. H. van Herk, director of the Laboratory for General Botany, Plant Physiology and Pharmacognosy, Amsterdam, for his valuable suggestions and constant interest during this investigation.

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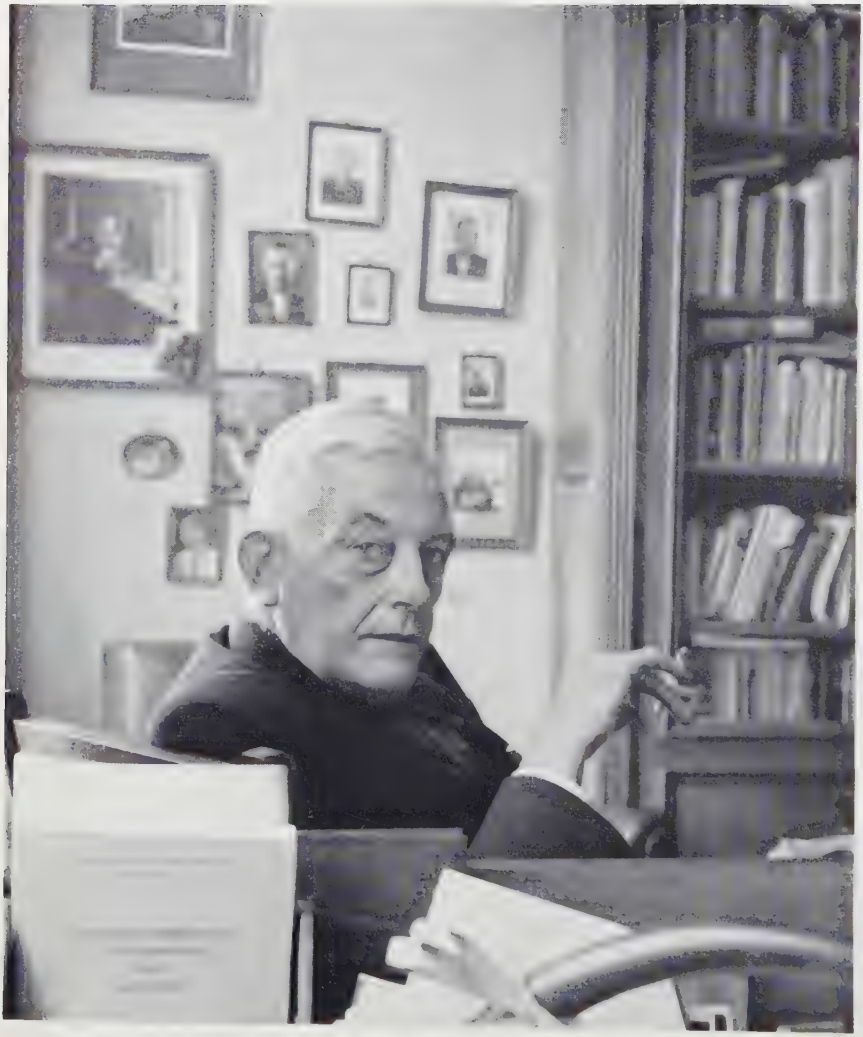

A. J. KLUYVER

IN MEMORIAM Prof. Dr. A. J. KLUYVER
1888–1956

Many scientists, and especially the numerous friends he had among them, were deeply moved when on the 14th May it became known that in the early hours of that day professor Dr. A. J. Kluyver had suddenly passed away. Only two days before Kluyver had attended the annual meeting of the "Hollandsche Maatschappij der Wetenschappen" at Haarlem. At the social gathering after the meeting he had keenly taken part in the discussion of scientific problems with several of his friends. Nobody at that time could think that his end was so near at hand.

Albert Jan Kluyver was born on the 3rd June 1888 at Breda. After his father had been appointed professor of mathematics at Leyden University, Kluyver followed the primary and secondary school in that town. From 1905–1910 he studied at the Technical University, Delft, where he graduated in chemical technology.

Kluyver, however, had acquired a great interest in botany, and he therefore accepted a post of assistant to professor G. van Iterson. This post he occupied for more than five years. In 1911 he spent a semester at Vienna to study plant physiology in von Molisch' laboratory, and in 1914 he obtained his doctor's degree on a thesis dealing with the biochemical determination of sugars. In a mixture of sugars the amount of each of them can be estimated by making use of the faculty of definite yeasts to ferment particular sugars.

In 1916 Kluyver went to Buitenzorg (Java) as research worker in the industrial sector of the Department of Agriculture, Industry and Commerce. In 1919–1920 he was sent to Ceylon and Malabar in order to study the coconut fibre industry in those countries and from 1920–1921 he acted as consultant to a private company engaged in the production of vegetable oils.

In those years he investigated not only the digestion of maltose by fungi, the alcoholic fermentation, and the biochemistry of fungi in general, but also the chemistry of chlorophyll and the action exercised by ultra-violet radiation on higher plants. For industrial purposes he studied the preparation and the properties of vegetable fibres and oils.

Kluyver therefore was not a specialized microbiologist when, in 1921, he was called to Delft to occupy the chair of microbiology thus far held so brilliantly by the late professor M. W. Beijerinck.

It is not unlikely that Kluyver's knowledge and experience of plant physiology and his general interest in biochemistry may

largely have contributed to the notable and varied achievements of KLUYVER and his students, the "Delft school" of microbiology. Although the course given by him was an optional one, KLUYVER nevertheless attracted quite a number of talented students, who in search of a subject for a doctor's thesis found inspiration in his teaching and started working in his laboratory.

With a prophetic view KLUYVER in 1926 indicated a way out of the troubled discussions on the nature of biological oxidations. In his paper "Die Einheit in der Biochemie" he advocated the view that all types of catabolic processes could be explained in terms of coupled catalytic dehydrogenations and hydrogenations. Later on, based on C. B. VAN NIEL's work on the photosynthesis of purple sulphur bacteria, KLUYVER and VAN NIEL extended this theory over the whole field of the metabolism. In the photosynthesis of the green plant the hydrogen needed for the reduction of carbon dioxide, would be supplied by water. So photosynthesis is not only the source of the organic matter wanted by non-green heterotrophic organisms, but it is in addition a mighty hydrolytic process, by wich the oxygen of the atmosphere has been produced, and in this way it created the conditions for aërobic life

As he had done several times before at other universities, KLUYVER, together with VAN NIEL, delivered in 1954 at Harvard University a series of lectures which recently have been published under the title "The Microbe's Contribution to Biology". This book gives an up to date survey of our knowledge of metabolism seen in the light of the hydrogenation-dehydrogenation theory.

The isolation, in a crystalline state, of the tobacco mosaic virus by W. M. STANLEY made a deep impression on KLUYVER's mind. This is clear from the address he delivered two years later at the 26th "Nederlandsch Natuur- en Geneeskundig Congres" (1937), under the suggestive title "'s Levens nevels" ("The foggy Borders of Life"). The fading of the sharp border between the living and the non-living, together with the striking unity he saw in the structure and chemistry of all life, since then strongly occupied him. He cherished the idea of a single unity in the whole of Creation. Especially in the last years he liked to discuss with his friends this Unity and Man's place and vocation in it. KLUYVER's last speech in the combined session of the "Koninklijke Nederlandse Akademie van Wetenschappen" (4th April 1955) on "Microbe en leven" ("Microbe and Life") may be considered a philosophical testimony and an expression of his personal creed.

KLUYVER's scientific activities were not confined to the field just mentioned. Since 1922 the collection of yeasts of the "Centraal Bureau voor Schimmelcultures" at Baarn (Director

Prof. JOH. WESTERDIJK) is kept in the Delft laboratory under KLUYVER's supervision and since that time several important monographs on yeasts were issued from there.

In 1937, sponsored by the Rockefeller Foundation, a biophysical research team was established at the Physical Laboratory of Utrecht University. The direction was entrusted to KLUYVER and the late professor L. S. ORNSTEIN. This team has published a number of papers dealing with photosynthesis and bioluminescence.

Of eminently practical importance (e.g. for preparing antibiotics) has become KLUYVER's method of "shaking" or "stirring" cultures of micro-organisms.

Finally it has to be mentioned that KLUYVER took an important share in scientific organisations and in the organisation of science. So he has been President of the "Koninklijke Nederlandse Akademie van Wetenschappen" (1947–1954), of which he has been a fellow for 30 years.

With KLUYVER's death science has lost one of its outstanding scholars. Yet, for his friends the loss of his particular personality is not less grave. KLUYVER's merits and authority received manifold recognition, and numerous were the honours bestowed upon him. Still he always remained a simple person, easy of access, humane in his criticism, cordial in his sympathy. In the meantime he was endowed with an innate dignity of style, which found expression in his scrupulous sense of responsibility as well as in his mastery over the language in which he expressed his views. It is reverence for this style which exhorts those who have known him, not to persist in mourning his death, but to keep him in mind as an ever shining example.

Utrecht, Botanical Laboratory V. J. KONINGSBERGER

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INFLUENCE OF LIGHT AND SUCROSE ON THE UPTAKE AND TRANSPORT OF CHLORIDE IN VALLISNERIA LEAVES

BY

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(*received July 17th, 1956*)

Introduction and method

The problem discussed in this publication is the influence of light and sucrose on uptake and transport of chloride ions. The system used for the experiments has been kept as simple as possible.

In order to study the uptake of chloride ions parts of leaves 2.5 cm long and 4 mm wide have been used in series of 8, absorbing chloride from solution or from agar. The series were kept in the dark or they were exposed to light, sugar or some other substance being added to the medium or not. The solutions were constantly being aerated with air free from carbon dioxide. The temperature amounted to 25° C. For examining the transport 5 cm leaflengths have been used in boxes of opaque black perspex, which were divided into two parts by a partition slotted for the leaflengths. The first zone of 2.5 cm of the leaflengths which is called the absorbing part in this paper is placed between agar strips or immersed in a solution containing KCl with $CaSO_4$ together with substances as sugars, mannitol and inositol, the influence of which on uptake and transport can be examined. The second 2.5 cm of the leaflengths, the free part, is placed in the adjoining compartment of the perspex box between agar plates or in a solution, if required with addition of other substances. The uptake from agar and the one from solution have always been used side by side for checking purposes. An advantage of the agar method is the local administration, the protection of the leaftissue from drying out, the proper aeration and the effective prevention of the contents of the two compartments from being mixed. In using fluids a passage through the slots in the partition is prevented by filling them up with vaseline mixed with carbon particles. The compartments can be covered with transparent or black perspex plates.

In experiments with closed perspex boxes the quantity of carbon dioxide present is rather small. Respiration produces also some carbon dioxide which can be used in photosynthesis. The quantity of carbohydrates synthesized in the boxes will be rather small especially if the solutions used are free from carbon dioxide and aerated with carbon dioxide free air; on the other hand it cannot be proved that they have had no influence at all on the accumulation processes.

For the last few years *Vallisneria* has been cultivated in a cellar with exposure to artificial light in concrete tanks filled with water purified by ion exchangers. The leaves poor in chloride can take up great quantities of chloride in the light. The agar used was Difco agar, specially purified for the purpose.[1])

It is our experience that agar raises the uptake of chloride somewhat. This may be attributed to carbo-hydrates being present. The preparation of the leaf material entirely corresponds with that of previous investigations. Before the experiments the leaves were well washed and from their sides so much was cut off that the width everywhere amounted to 4 mms. Next they were cut with a sharp knife into smaller pieces of 2.5 or 5 cms and put in distilled water. Eight series are composed from 8 identical leaf pieces taken from 8 different leaves and from different parts of these leaves. The pieces of one series are arranged in a perspex frame so that they don't overlap. Before the uptake begins the leaf segments in their frame remain in aerated distilled water for 24 hours in order to eliminate the wound effect caused by the cutting.

I. *Influence of light and sucrose on the uptake in 2.5 cm leaf segments*

The uptake of chloride in the dark varies a great deal in strength. Per series it may amount from 0 to more than 200 μg which depends on the pretreatment, particularly the exposure and the supply of carbohydrates. By addition of sucrose to the medium the uptake may be increased considerably (Table 1). The material of these experiments

TABLE 1

Influence of sucrose and light on the uptake of chloride. Pretreatment 24 hours' exposure to light. Uptake from 1/1000 M KCl + $CaSO_4$ solution. The molarity relates to the KCl. In exp. 835 uptake from agar with 1/500 M KCl + $CaSO_4$. Instead of sucrose 1/20 M 2 % fructose has been added to the agar. In all tables uptake is given in μg Cl per 8 leaf segments of 2.5 cm length and 0.4 cm width per 24 hours at 25°C.

	Exp. 1004	Exp. 1006	Exp. 1007	Exp. 1086	Exp. 835
dark	99	78	110	153	36
dark + sucr. 1/20 M	256	220	195	185	43
light — CO_2	568	401	461	469	193
light + sucr. 1/20 M	618	504	561	540	267

showed a rather strong uptake in the dark of ± 100 μg. By administration of sucrose 1/10 M it rises to 256 μg.

The material was pretreated at 100 f.c. white light for 24 hours. Exposure during the uptake to 100 f.c. has a stronger effect than administration of sucrose. During the exposure air free from carbon dioxide was led through the solution. This causes an increase of pH and a disappearance of CO_2 and bicarbonate from the solution. It

[1]) We acknowledge our indebtedness to the Difco Laboratories Detroit (Mich). for their help in providing the purified agar and to the Wester Suikerraffinaderij for placing purified sucrose at our disposal.

may be presumed that in this way the formation of carbo-hydrates in the photosynthesis is for the most part inhibited (ARISZ 1947).

If besides exposure sucrose is added, the uptake becomes greater yet. Fructose has an identical influence as sucrose (Table 1). Also mannitol and inositol stimulate the uptake of chloride in light and dark (Table 2).

From Table 3 it appears that addition of sucrose to about 0.24 M

TABLE 2

Influence of inositol on the chloride uptake from 1/1000 M KCl + $CaSO_4$

	In the light	In the dark
24 hours uptake	504	43
,, ,, with 0.04 M inositol	513	43
,, ,, ,, 0.06 ,, ,,	563	46
,, ,, ,, 0.09 ,, ,,	589	60
,, ,, ,, 0.15 ,, ,,	660	71

TABLE 3

Influence of sucrose on the uptake of chloride in the light. Pretreatment 24 hours in the light. Uptake 24 hours from 1/1000 M KCl + $CaSO_4$. (Exp. 900)

24 hours uptake from 1/1000 M KCl + $CaSO_4$	245
with addition of 0.02 M sucrose	308
0.04 ,, ,,	318
0.08 ,, ,,	353
0.16 ,, ,,	370
0.24 ,, ,,	391
0.32 ,, ,,	*184*

results in an increase of the uptake of chloride ions in the light. At a still higher sugar concentration, however, the uptake decreases, because at ± 0.3 M sucrose plasmolysis takes place. As the protoplasts are separated by plasmolysis, it is comprehensible that the uptake into and the transport to the cells not adjoining the medium greatly decrease. In that case a symplasmatic transport is of course no more possible. This sets a limit to the concentration of sucrose, from which a favourable effect on the uptake may be expected.

It may be that the sugars glucose, fructose and sucrose behave differently. They are taken up into the cells, where they are converted. One sugar is transported better than another. The differences, however, have not been fully investigated.

To discover whether the influences of light and sucrose are of the same nature the following experiment has been made. In it we started from the notion, that if the influence of sugar and of light are identical, a further addition of sucrose will not have any influence on the salt uptake with light saturation. For this purpose *Vallisneria* material has been pretreated in the dark for 24 hours and next exposed to 150, 200 and 300 f.c. white light for 24 hours chloride uptake (Table 4 experiment 1089). It appears that already at 150 f.c. light saturation has set in. Addition of 1/10 M sucrose, however, results as well at 150, at 200 as at 300 f.c. in a further uptake of more than 100 μg Cl.

This indicates that the influence of exposure and addition of sugar cannot be based on the primary formation of one and the same substance (cf. p. 243).

TABLE 4

Influence of exposure to light and sucrose addition on the uptake of chloride. Pretreatment 5 hours in the dark. Uptake from a 1/1000 M KCl + $CaSO_4$ solution during 24 hours; aerated with carbondioxide free air. (Exp. 1089)

dark	186
dark + sucrose	232
exposure 150 f.c.	264
,, 150 f.c. + sucrose	384
,, 200 f.c.	267
,, 200 f.c. + sucrose	370
,, 300 f.c.	257
,, 300 f.c. + sucrose	367

II. *Influence of exposure to light of the absorbing zone in 5 cm leaf lengths*

In Table 5 data have been gathered on the influence of exposure of the absorbing zone on the accumulation of chloride ions in the absorbing and in the free leaf part. Two cases are to be distinguished: the free zone of the leaf may be exposed to light or kept in the dark.

Only a few experiments will be discussed more fully. Experiment 1087 is remarkable. In series 3 the absorbing and the free part are both in the dark, sucrose being added to both. On comparison with series 1 of the same experiment this addition of sucrose to the free part in the dark appears to have resulted in a slight promotion of the uptake. If the absorbing part is exposed (series 4) the accumulation in the absorbing part increases from 160 to 408 μg, that in the free part from 63 to 178 μg. So the total uptake increases from 223 to 586 μg. It is interesting that in this case the free part also accumulates more and that only on exposure of the absorbing part the stimulating action of the sucrose in the free part has a stronger effect.

From this it appears that exposure of the absorbing part increases the supply of ions to the symplasm and makes a stronger accumulation possible both in the absorbing and in the free part.

In experiment 1014 series 1 and 2 exposure of the absorbing part increases the accumulation in the free part in the dark from 82 to 146 μg, while in the absorbing part itself the accumulation increases from 117 to 373 μg.

Exposure of the absorbing zone, the free zone being in the light (experiment 1014 series 3 and 4) likewise results in an increase of accumulation in both zones; in the absorbing zone there is an increase of 142 to 475 μg, in the free zone from 46 to 280 μg. Here therefore the accumulation in the free part is restricted by the slight uptake in the absorbing zone which is in the dark. This indicates that exposure to light raises the chloride ion concentration in the symplasm and that as a result the accumulation in the free part in the dark, in the light and in the dark with sucrose increases. Experiment 1026 gives the same picture. Experiments 1024 and 1025 show a bad transport,

TABLE 5

Influence of exposing the absorbing part to light. Pretreatment with 1/20 M fructose in the light (1025 and 1026), with sucrose (1024), others water. Uptake from agar with 1/100 M KCl + $CaSO_4$, S 41 from 0.002 M KCl solution. Addition of 1/20 M sucrose; 24 hours.

series	exposure	Exp. 1014	Exp. 1024	Exp. 1025	Exp. 1026
1	dark — dark	117 — 82 (199)	103 — 3 (100)	110 — 3 (107)	146 — 56 (202)
2	*light* — dark	**373** — **146** (519)	**199** — 7 (192)	**200** — **66** (266)	**454** — 46 (500)
3	dark — light	142 — 46 (188)	121 — 7 (114)	102 — 67 (169)	156 — 78 (234)
4	*light* — light	**475** — **280** (755)	**197** — **35** (232)	**334** — **110** (444)	**589** — **295** (884)

		Exp. 1087		Exp. 1088
1	dark + sucr. — dark	127 — 53 (180)	dark + sucr. — light	250 — 44 (294)
2	*light* + sucr. — dark	**284** — **100** (384)	*light* + sucr. — light	**350** — **55** (405)
3	dark + sucr. — dark + sucr.	160 — 63 (223)	dark + sucr. — light + sucr.	263 — 51 (314)
4	*light* + sucr. — dark + sucr.	**408** — **178** (586)	*light* + sucr. — light + sucr.	**392** — **69** (461)
5	dark — dark + sucr.	120 — 63 (183)	dark — light + sucr.	239 — 51 (290)
6	*light* — dark + sucr.	**252** — **114** (366)	*light* — light + sucr.	**321** — 44 (365)

TABLE 5A

Localising effect of sucrose

	Exp. S 41
dark — light	169 — 89 (258)
light — light	**344** — **181** (525)
dark + sucr. — light	238 — 96 (334)
light + sucr. — light	**451** — 96 (547)

as has repeatedly been found in *Vallisneria* leaves. Yet the effect of exposure of the absorbing part is noticeable here too in a stronger accumulation in the free part. In experiment 1088 transport is also slight in all circumstances. Here again it is a different factor, the conductivity of the symplasm that keeps the transport on a low level and as a result limits the accumulation in the free part.

From these experiments it follows that on uptake in the dark the accumulation in the free zone is limited by the supply of chlorides in the symplasm of the absorbing part.

Exposure of the absorbing part increases the supply of ions to the symplasm of the absorbing part and by doing so it raises the accumulation both in the absorbing and in the free part.

Just as the experiments with inhibitors mentioned in a previous publication (Arisz 1953, 1956) and the experiments on redistribution (1954, 1956) these experiments are a proof of the fact that in Vallisneria leaves the transport of chloride ions to the free part of the leaf takes place in a symplasm and not in the cell walls. For in that case an exposure to light of the absorbing zone could never have such a decisive influence on the accumulation in the free part.

III. *Influence of exposure to light of the free zone*

III A. Two cases are to be distinguished. In the first the absorbing part is in the light (Table 6). In this case exposure of the free part as a rule causes an increase in accumulation in this zone, which may sometimes be considerable (16 times an increase has been found, once a slight decrease). In the presence of sucrose the accumulation in the dark is mostly fairly great in the free zone, so that the influence of exposure grows less distinct. It is, however, remarkable, that exposure of the free part sometimes causes a considerable increase up to 50 % or more of the accumulation in the absorbing part (Exp. 1010 series 3 and 4, 5 and 6, exp. 1083 series 7 and 8, exp. 1014, 1025 and 1026). In experiment 1112 the free part of the leaf was in distilled water. During exposure to light it was aerated with carbon dioxide free air. This means that during exposure formation of carbo-hydrates was for the greater part prevented. *This is an important observation which shows that from the exposed free part there proceeds an influence on the absorbing zone, which increases the accumulation there in spite of the absorbing part already being in the light.* So exposure to light of the free zone has two results: a stronger accumulation on the spot and a stronger accumulation in the absorbing zone (Table 21).

III B. In the second case the absorbing part is left in the dark. (Table 7). Here again exposure of the free zone results as a rule in an increase of the accumulation in the absorbing and in the free zone provided sucrose has been administered to the absorbing and to the free zone at the same time. If, however, the absorbing zone is in the dark and does not get any sucrose the effect is variable (Table 7). As a rule the accumulation in the absorbing part increases, while the chloride ion concentration in the free part also increases, but in

TABLE 6

Influence of exposure to light of the free part, the absorbing part being in the light. Uptake from agar with 1/100 M KCl + $CaSO_4$; addition of 1/20 M sucrose, 24 hours.

	Exp. 1010	Exp. 1083	Exp. 1013	Exp. 1112
light — dark	351 — 138 (489)	209 — 50 (259)	366 — 89 (455)	160 — 75 (235)
light — *light*	**376** — **185** (561)	**263** — **131** (394)	**401** — **209** (610)	**196** — **92** (288)
light — dark + sucr.	355 — 135 (490)	273 — 117 (390)		189 — 116 (303)
light — *light* + sucr.	**508** — **238** (746)	**316** — **135** (451)		196 — **153** (349)
light + sucr. — dark	413 — 138 (551)	228 — 107 (335)		227 — 96 (323)
light + sucr. — *light*	**497** — **160** (657)	235 — **135** (370)		**252** — **167** (419)
light + sucr. — dark + sucr.	462 — 281 (743)	238 — 110 (348)		284 — 128 (412)
light + sucr. — *light* + sucr.	**511** — 241 (752)	**341** — **128** (469)		**352** — **210** (562)
	Exp. 1024	Exp. 1025	Exp. 1026	Exp. 1014
light — dark	199 — – 7 (192)	200 — 66 (266)	454 — 46 (500)	373 — 146 (519)
light — *light*	197 — **35** (232)	**334** — **110** (444)	**589** — **295** (884)	**475** — **280** (755)

TABLE 7

Influence of exposure to light of the free part, the absorbing part being in the dark. Uptake from 1/1000 M KCl + $CaSO_4$ solution (1004, 1006 and 1007), S 18, S 19 and S 21 from 0.002 M solution; from agar with 1/100 M KCl + $CaSO_4$ (1014, 1024, 1025, 1026).

	Exp. 1004	Exp. 1006	Exp. 1007	Exp. 873	S 21 L	Exp. 1094
dark — dark	121 — 125 (246)	128 — 64 (192)	71 — 14 (85)		64 — 7 (71)	82 — 18 (100)
dark — *light*	**135** — *7 (142)*	*85* — *43 (128)*	**85** — **35** (120)		**103** — 10 (113)	**153** — **75** (**228**)
dark + sucr. — dark	156 — 50 (206)	206 — 50 (256)	199 — 64 (263)	49 — 3 (52)		139 — 25 (164)
dark + sucr. — *light*	**224** **71** (295)	**263** — 57 (**320**)	**220** — **85** (**305**)	**158** — **28** (**186**)		**202** — **110** (**312**)

	Exp. 1024	Exp. 1025	Exp. 1026	Exp. 1014	S 18H	S 19H
dark — dark	103 — −3 (100)	110 — −3 (107)	146 — 56 (202)	117 — 82 (199)	67 — 3 (70)	99 — 14 (113)
dark — *light*	**121** - 7 (114)	102 — **67** (**169**)	**156** — **78** (**234**)	**142** — *46 (188)*	63 — **56** (**119**)	**116** — **99** (**215**)

TABLE 7A

	Exp. 1118
dark — dark	139 — 50 (189)
dark — *light*	**178** — **86** (264)
dark + sucr. — dark	150 — 57 (207)
dark + sucr. — *light*	**228** — **103** (331)
dark — dark + sucr.	143 — 72 (215)
dark — *light* + sucr.	**188** — **114** (302)
dark + sucr. — dark + sucr.	153 — 86 (239)
dark + sucr. — *light* + sucr.	**249** — **121** (370)

exp. 1004 series 2 and 1006 series 2 it decreases. This phenomenon has only been stated a few times.

In Table 7 A (exp. 1118) the exposed free part was in distilled water and aerated with carbon dioxide free air. In this experiment the influence of the formation of carbo-hydrates in the light was for the greater part prevented. The increased accumulation in the absorbing zone is the result of a specific substance formed in the exposed free part and transported to the absorbing zone.

In previous experiments (1947, 1948 and 1953) on material cultivated in the day light, we repeatedly found that the accumulation in the exposed free zone was greater than in the absorbing part that was kept in the dark. As an example we give Table 8 (cf. ARISZ 1947 Table 7 and 1953 figs. 8A and 11A). This may be explained by assuming that

TABLE 8

Influence of exposing the free part of the leaf to light.

	Exp. 832
A. Uptake from 1/500 M KCl + $CaSO_4$ solution in the light	75
Uptake from 1/500 M KCl + $CaSO_4$ solution in the light + 2 % fructose	150
Absorbing part in the dark + fructose, free part in the light + fructose	29 — 133 (162)
	Exp. 821
B. Absorption by a 2.5 cm leaf segment in the dark + 2 % fructose	30
Absorption by a 2.5 cm leaf segment in the light + 2 % fructose	128
5 cm leaf segment; absorbing part in the dark + 2 % fructose / free part in the dark + 2 % fructose	17 — 7 (24)
5 cm leaf segment; absorbing part in the dark + 2 % fructose / free part in the light + 2 % fructose	65 — 57 (122)

in this case transport is fast in the symplasm so that with the restricted uptake in the dark, the accumulation in the absorbing part is held down in favour of that in the free part. Scarcity of carbo-hydrates in the absorbing zone may promote this. In Table 8B the uptake in the dark of a 2.5 cm leafsegment in the presence of fructose is 30 μg, through exposure it increases to 128 μg. If the absorbing and the free zone of a 5 cm leaflength are both in the dark, fructose being present in the medium, there is hardly any accumulation in the free zone (7 μg). If, however, the free part of a 5 cm leaflength is exposed to light, we find a total uptake of 122 μg, apportioned between 65 μg in the absorbing and 57 μg in the free zone. In this case exposure of the free zone caused a rise in the total increase from 24 to 122 μg. This is fairly equal to the uptake of a 2.5 cm leaflength exposed to light. Here therefore the effect of an exposure of the free zone on the total uptake is equally great as the effect of an exposure on the uptake of

a segment of 2.5 cm length. This also shows an influence of exposure of the free zone on the uptake from the medium.

IV. *Influence of addition of sucrose to the absorbing part in the light*

IV A. We shall now discuss experiments in which the influence of *sucrose* has been investigated. This may be administered to the absorbing or to the free zone, while moreover the prevailing exposure may have an influence on the effect.

Table 9 contains data in the case the free zone is in the light or in the dark, sucrose being present or not. The result is clear. There always occurs an increase of accumulation in the absorbing zone; there is an increase in the free part in 10 of the 17 exp., but in other cases the latter continues unaltered. In exp. 1010 and S 41 there is a decrease. In most cases the total uptake increases considerably.

IV B. Remarkable is the influence of adding sucrose to the absorbing part, while it is in the dark (Table 10). The total uptake of Cl increases, but less so than if the absorbing zone is exposed to light. The accumulation in the absorbing part always increases. The results concerning the accumulation in the free part, however, vary in this case. In the 26 experiments eleven times a decrease, eight times an increase was found; in the other cases there was no change. This tendency to a decrease of accumulation in the free part must be due to the increased competition exercised by the plasm adjoining the vacuoles in the absorbing part. It accumulates chloride ions from the symplasm into the vacuoles, owing to which fewer chloride ions remain available for transport to the free part. Though by the sucrose the total uptake of chloride ions from the medium is increased there is a competition between the accumulation in the absorbing and that in the free part, since the uptake is restricted in the dark.

V. *Influence of addition of sucrose to the free part*

Table 11 gives the results of administering sucrose to the free part, the absorbing zone being exposed to the light. The promoting influence of sucrose is as a rule small and local, but in some cases there is an increased accumulation in the absorbing part too. In experiment 1083 the accumulation in the absorbing zone decreases in 2 cases, in two others it increases. The differences are not great in this experiment. The total uptake remains equal in many cases, but it may sometimes increase.

Table 12 contains the results of 23 observations on the influence of administering sucrose to the free leaf zone, the absorbing zone being in the dark. It is a striking fact that in eleven cases the total uptake (accumulation in absorbing and free parts together) decreases strongly whereas in three it remains fairly equal and in nine increases slightly. Especially if the absorbing zone is in the dark without a supply of sucrose, the phenomenon occurs that on sucrose addition to the free part, the chloride accumulation in the absorbing part diminishes in

TABLE 9

Influence of sucrose addition to the absorbing part, the free part being in the light. Uptake from agar with 0,01 M KCl + $CaSO_4$, S 41 from a 0.002 M solution; addition of 0.05 M sucrose, 24 hours.

	Exp. 1013	Exp. 1010	Exp. 1083	S 41	Exp. 1112
light — light	401 — 209 (610)	376 — 185 (561)	263 — 131 (394)	344 — 181 (525)	196 - 92 (288)
light + *sucr.* — light	**586** — **366** (952)	**497** — *160* (657)	**316** — 135 (451)	**451** — *96* (547)	**252** — **167** (419)
				Exp. 1088	
light — light + sucr.	401 — 259 (660)	508 — 238 (746)	235 — 135 (370)	321 — 44 (365)	196 — 153 (349)
light + *sucr.* — light + sucr.	**586** — **486** (1072)	511 — 241 (752)	**341** — 128 (469)	**392** — **69** (461)	**352** — **210** (562)
light — dark		351 — 138 (489)	209 — 50 (259)		160 — 75 (235)
light + *sucr.* — dark		**413** — 138 (551)	**273** — **117** (390)	Exp. 1087	**227** — **96** (323)
light — dark + sucr.		355 — 135 (490)	228 — 107 (335)	252 — 114 (366)	189 — 116 (305)
light + *sucr.* — dark + sucr.		**462** — **281** (743)	238 — 110 (348)	**408** — **178** (586)	**284** — **128** (412)

TABLE 10

Influence of sucrose addition to the absorbing part, the free part being in the dark. Uptake from agar with 0.01 M KCl + $CaSO_4$ (1087, 1088), 0.002 M KCl + $CaSO_4$ (873, 874, 878, 879), from 0.001 M KCl + $CaSO_4$ solution (1004, 1006, 1007), from 0.002 M solution S 8 and S 9; 24 hours.

	Exp. 873	Exp. 874	Exp. 878	Exp. 879	S 9H	S 8L
dark — light	98 — 46 (144)	88 — 60 (148)	80 — 87 (167)	53 — 84 (137)	57 — 50 (107)	131 — 17 (148)
dark + *sucr.* — light	**221** — *21* (242)	**197** — *46* (243)	**186** *45* (231)	**76** — *53* (129)	**85** — *14* (99)	**258** — *11* (269)
dark — light + sucr.	53 — 67 (120)	35 — 53 (88)	102 - 68 (170)	30 — 37 (67)	7 — 64 (71)	116 — 17 (133)
dark + *sucr.* — light + sucr.	**158** — *28* (186)	**151** — *21* (172)	**186** — **102** (288)	**91** — **64** (155)	**75** — *50* (125)	**180** — - *3* (177)
	Exp. 1087					Exp. 1118
dark — dark + sucr.	120 — 63 (183)					143 — 72 (215)
dark + *sucr.* — dark + sucr.	**160** — 63 (223)					**153** — **86** (239)
		Exp. 1004	Exp. 1006	Exp. 1007	Exp. 1094	
dark — light		135 — 7 (142)	85 — 43 (128)	85 — 35 (120)	153 — 75 (228)	178 — 86 (264)
dark + *sucr.* — light		**224** - - **71** (295)	**263** **57** (320)	**220** — **85** (305)	**202** — **110** (312)	**228** — **103** (331)
	Exp. 1088					
dark light + sucr.	239 — 51 (290)	7 - 106 (113)	156 — 99 (255)	7 — 104 (111)		188 — 114 (302)
dark + *sucr.* — light + sucr.	**263** — 51 (314)	**248** 99 (347)	**241** — 99 (340)	**213** — *85* (298)		**249** — 121 (370)
dark — dark					82 — 18 (100)	139 — 50 (189)
dark + sucr. — dark					**139** — 25 (164)	**150** — 57 (207)

TABLE 11

Influence of sugar addition to the free part, the absorbing part being in the light. Uptake from agar with 0.01 M KCl + $CaSO_4$ 24 hours, sucrose 0.05 M.

	Exp. 1013	Exp. 1010	Exp. 1083	Exp. 1088	Exp. 1112
light — light	401 — 209 (610)	376 — 185 (561)	263 — 131 (394)		196 — 92 (288)
light — light + *sucr.*	401 — **259** (660)	**508** — **238** (746)	*235* — 135 (370)		196 — **153** (349)
light — dark		351 — 138 (489)	209 — 50 (259)		160 — 75 (235)
light — dark + *sucr.*		355 — 135 (490)	**228** — **107** (335)		**189** — **116** (305)
light + sucr. — light	586 — 366 (952)	497 — 160 (657)	316 — 135 (451)	350 — 55 (405)	252 — 167 (419)
light + sucr. — light + *sucr.*.	586 — **486** (1072)	511 — **241** (752)	**341** — 128 (469)	**392** — **69** (461)	**352** — **210** (562)
				Exp. 1087	
light + sucr. — dark		413 — 138 (551)	273 — 117 (390)	284 — 100 (384)	227 — 96 (323)
light + sucr. — dark + *sucr.*.		**462** — **281** (743)	*238* — 110 (348)	**408** — **178** (586)	**284** — **128** (412)

TABLE 12

Influence of sugar addition to the free part, the absorbing part being in the dark. Uptake from agar with 0.002 M KCl + $CaSO_4$ (873, 874, 878, 879), from agar with 0.01 M KCl + $CaSO_4$ (1087, 1088), from 0.001 M KCl + $CaSO_4$ solution (1004, 1006, 1007); S 7, S 8, S 9 from 0.002 M solution.

	Exp. 873	Exp. 874	Exp. 878	Exp. 879	Exp. 1004	Exp. 1006	Exp. 1007
dark — light	98 — 46 (144)	88 — 60 (148)	80 — 87 (167)	53 — 84 (137)	135 — 7 (142)	85 — 42 (127)	85 — 35 (120)
dark — light + *sucr.*.	*53* — **67** (*120*)	*35* — 53 (*88*)	**102** — *68* (170)	*30* — *37* (*67*)	*7* — **105** (*113*)	**156** — **99** (**255**)	*7* — **104** (111)
dark + sucr. — light	221 — 21 (242)	197 — 46 (243)	186 — 45 (231)	76 — 53 (129)	224 — 71 (295)	263 — 57 (320)	220 — 85 (305)
dark + sucr. — light + *sucr.*.	*158* — 28 (*186*)	*151* — *21* (*172*)	186 — **102** (**288**)	**91** — 64 (**155**)	**248** — **99** (**347**)	*241* — **99** (**340**)	213 — 85 (298)
	Exp. 901	S 8L	S 7H	S 9H			
dark — light	126 — 36 (162)	131 — 17 (148)	103 — 111 (214)	57 — 50 (107)			
dark — light + *sucr.*.	*66* — **57** (*123*)	*116* — 17 (*133*)	*11* — *60* (*71*)	*7* — **64** (*71*)			
	Exp. 1088						
dark + sucr. — light	250 — 44 (294)	258 — 11 (269)	46 — 4 (50)	85 — 14 (99)			
dark + sucr. — light + *sucr.*.	**263** — 51 (**314**)	*180* — *3* (*183*)	*4* — **89** (**93**)	*75* — **50** (**125**)			
	Exp. 1087						
dark + sucr. — dark	127 — 53 (180)						
dark + sucr. — dark + *sucr.*.	**160** — **63** (**223**)						

9 of the 11 cases. The accumulation in the free part is rather variable, it either increases or decreases.

If sucrose has been administered to the absorbing part a decrease of the accumulation in that zone occurs in 6 cases out of 12, in four cases there is an increase, in two no change. The total quantity accumulated in the two zones together increases in 8 and decreases only in 3 cases. This indicates that administration of sucrose to the absorbing part diminishes the long distance effect of the sugar addition to the free part.

This phenomenon of a long distance effect of sucrose addition to the free part on the accumulation in the absorbing part was quite unexpected and has therefore been carefully investigated.

Mrs. Knobbe and Miss Schreuder made observations about this phenomenon in the years 1953 and 1955. In order to eliminate the influence of the person who makes the determinations, the experiments have been repeated by the junior author (exp. marked S). The phenomenon appeared to have a good reproducibility. It is not so variable as some other reactions of *Vallisneria*, which are greatly dependent on the nutritional condition. It has appeared (Arisz 1955; Arisz and Schreuder 1956) that this phenomenon has an *osmotic cause*. Table 13 contains two experiments pointing in this direction,

TABLE 13

Influence of sucrose concentration added to the free part, the absorbing part being in the light. Uptake from agar with 0.002 M KCl + $CaSO_4$ (899) from 0.002 M KCl + $CaSO_4$ solution (S 15).

	Exp. S 15L	Exp. 899
dark — light	**103** — 3 (106)	72 — 38 (110)
dark — light + 0.005 M sucr.	**117** — 39 (156)	
dark — light + 0.020 M sucr.	*88* — 17 (105)	
dark — light + 0.040 M sucr.		*45* — 24 (69)
dark — light + 0.060 M sucr.	*74* — 17 (91)	*31* — 17 (48)
dark — light + 0.090 M sucr.		*31* — 21 (52)
dark — light + 0.100 M sucr.	*46* — 3 (49)	
dark — light + 0.150 M sucr.		*26* — 45 (71)

in which the influence of the concentration of the sucrose solution has been examined. It appears that with increasing sucrose concentration administered to the free part, the accumulation in the absorbing zone decreases.

In Table 14 sucrose in an increasing concentration has been added to the absorbing part, the same sucrose concentration 0.1 M being administered to the free part in all experiments. It appears that according as the sucrose concentration administered to the absorbing part increases the uptake increases too. At 0.1 M added both to the absorbing and to the free part of the leaf the accumulation in the absorbing and in the free zone is again normal and even a little higher than without sucrose being administered. Then sucrose again promotes accumulation.

It may be understood that if to the absorbing part itself sucrose is administered or if it has been placed in the light, the accumulation of chloride ions in the absorbing zone is promoted and the osmotic value of these cells increases. In that case the osmotic action of the

TABLE 14

Influence of increasing the sucrose conc. administered to the absorbing part on the osmotic effect of a sucrose solution in contact with the free part. Uptake from 0.002 M KCl + $CaSO_4$ solution

	Exp. S 23H	Exp. S 45
dark — light	**88** — 3 (91)	
dark — light + 0.1 M sucrose	*17* — 24 (41)	
dark + *0.02* M sucr. — light + 0.1 M sucrose	38 — 52 (90)	
dark + *0.06* M sucr. — light + 0.1 M sucrose	52 — 45 (97)	
dark + *0.10* M sucr. — light + 0.1 M sucrose	**95** — 52 (147)	
dark — light		96 — 32 (128)
dark — light + 0.05 M sucrose		*53* — **53** (106)
dark + *0.02* M sucr. — light + 0.05 M sucrose		**117** — **67** (184)
dark + *0.05* M sucr. — light + 0.05 M sucrose		**167** — 67 (234)
dark + *0.10* M sucr. — light + 0.05 M sucrose		**202** — **74** (276)

sucrose in the free part cannot present itself. Sucrose administered to an absorbing leaf zone has not, as we have seen, an inhibitory, but a promoting effect on the uptake of chloride ions, as long as plasmolysis does not take place (Table 3).

VI. *Influence of exposure and addition of sugar during pretreatment on the uptake of chloride ions*

The influence of a pre-exposure was already investigated before (Arisz 1947, p. 1028). It was then demonstrated that exposure during pretreatment increases the next following uptake of chloride. Aeration during the pretreatment with carbon dioxide free air and the withdrawal of carbon dioxide formed by the tissue does not prevent this favourable influence on the following uptake. So this effect has nothing to do with photo-synthesis of carbo-hydrates.

The intensity of the pre-exposure has a great influence.

In the experiments on influence of sucrose on uptake and transport the influence of sucrose on the strength of the chloride uptake has appeared. This gave rise to our bringing the influence of sucrose during pretreatment into our investigation.

Table 15 gives the result of three experiments which have been made on the influence of sucrose and light during pretreatment. The pretreatment took place in the dark, in the dark with addition of 0.1 M sucrose, in light of 150 f.c. in the absence of CO_2 and in light of this intensity with addition of 0.1 M sucrose. In experiment 1091 the uptake took place in two different light intensities, 50 and 150 f.c. in the absence of CO_2. In experiments 1100 and 1102 the uptake was determined in the dark and at 50 f.c. in the absence of CO_2. All determinations have been made in duplicate.

The uptake in the light at 50 and 150 f.c. is increased during the pretreatment by addition of sucrose. Pre-exposure without sucrose in a medium free from CO_2 causes a stronger increase in uptake than addition of sucrose and this light influence is dependent on the intensity

TABLE 15

Influence of exposure to light and of sucrose during the pretreatment on the uptake of chloride by Vallisneria leaves. In exp. 1091 exposure during uptake 50 and 150 f.c. in CO_2 free medium. In exp. 1100 and 1102 uptake in the dark with exposure to 50 f.c. in CO_2 free medium.

pretreatment 20 hours	uptake μg Cl 50 f.c. — CO_2	uptake μg Cl 150 f.c.—CO_2	uptake μg Cl dark		uptake μg Cl 50 f.c. — CO_2	
	Exp. 1091	Exp. 1091	Exp. 1100	1102	Exp. 1100	1102
dark	174	280	71	75	181	362
dark+0.1 M sucrose	309	366	117	117	359	451
light 150 f.c. — CO_2	401	444	220	138	380	540
light+0.1 M sucrose	444	525				

of the light during the pretreatment. In experiment 1091 addition of sucrose during pre-exposure has not a great, but yet distinctly favourable effect. From experiments 1100 and 1102 it follows that the influence of the pretreatment with sucrose and light is likewise noticeable if the uptake of chloride takes place in the dark. This is an important observation, to which we will revert (cf. fig. 3 curves A, B and C).

At the same time it appears from this experiment, which fact has been corroborated by further experiments, that a substance formed in the light can be distinguished from a substance formed in the dark in the presence of sucrose. It is clear that the sucrose is absorbed by the leaf during pretreatment, but what conversion it undergoes in the leaf is unknown. So we distinguish a preformed 'light substance' and a preformed 'sucrose substance'.

It was interesting to know whether the substance formed in the light is transported in the leaf. For this purpose experiments have been made (Table 16), in which one half of the 5 cm leaflengths in perspex boxes was exposed during pretreatment with 150 f.c. in the absence of CO_2, the other half remaining in the dark. It is of course impossible to ascertain that the exposed part did not have any CO_2 at its disposal. Communication of the two compartments was avoided as much as possible, but cannot be quite excluded, the filling up being done with vaseline and carbon particles. Besides there are big intercellular canals with septa in the leaf, so that respiration CO_2 in an exceedingly low concentration may have had some influence. The quantity of sugar, however, which might be formed in this way by photosynthesis is so small and the possibility of its being transported in the leaf to the adjoining part that remains in the dark, so slight, that this possibility may be left out of consideration. In experiment 1099 the exposed compartment was moreover aerated with CO_2 free air.

The 5 cm control leaflengths remained entirely in the dark during

pretreatment. After the pretreatment the 5 cm leaflengths were cut into two equal parts and in the next following uptake from 1/1000 M KCl + $CaSO_4$ exposed, one series to 50, a second series to 150 f.c. Three experiments have been made.

TABLE 16

Influence of pretreatment on the uptake of chloride. Transport of the substance formed during pretreatment in the light. Leaflengths of 5 cm pretreated dark-light or dark-dark. Exposure to 150 f.c. in carbondioxide free water. After the pretreatment the leaflengths have been cut in two parts of 2.5 cm. Uptake by the separated leafsegments in 50 and 150 f.c. from a 0.001 M KCl + $CaSO_4$ solution (μg Cl/24 hours, 8 segments of 2.5 cm)

pretreatment 5 cm leaflengths	2.5 cm parts	uptake	exp. 1092	exp. 1093	exp. 1099
$dark_1$ — light	$dark_1$	50 f.c.	277	284	210
		150 f.c.	309	323	249
	light	50 f.c.	408	369	405
		150 f.c.	430	412	462
$dark_2$ — $dark_3$	$dark_2$	50 f.c.	224	209	153
		150 f.c.	217	227	227
	$dark_3$	50 f.c.	209	209	156
		150 f.c.	217	217	231

The leafzone d 1 which was connected with the exposed zone during pre-exposure, had to be compared during the uptake with the leafsegments d 2 and d 3, which remained in the dark during pre-exposure. It appears from all three experiments that both at 50 f.c. and at 150 f.c. d 1 takes up more chloride than the average amount of d 2 and d 3.

In experiment 1092 this amounts for 50 f.c. to 277 against 216 μg, for 150 f.c. 309 against 217 μg. In experiment 1093 it amounts for 50 f.c. to 284 against 209 μg and for 150 f.c. 323 against 222 μg. In experiment 1099 for 50 f.c. 210 against 155 μg and for 150 f.c. 249 against 229 μg. The experiments make the impression that the substance formed in the light during pretreatment is transportable.

A fairly large number of experiments have been made on the influence of pre-exposure on uptake at various light intensities. In these experiments quite different results have been obtained, which may be understood now that it is known that the sugar condition of the leaves before the experiment also affects the uptake. The experiments cannot be very well compared with each other, as they have been carried out with different plant material. We shall discuss only one of these experiments, in which the influence of pretreatment in the dark and of pre-exposures to 70 and 120 f.c. have been compared. A Philips sodium lamp was used provided with a water filter to remove the heat rays. The uptake of 1/1000 M KCl + $CaSO_4$ was determined in the dark at 20, 40, 70, 120, 160 and 240 f.c. On account of the great number of points that had to be determined in one experiment, these observations could not be made in duplicate. The result of the pre-exposure is clear, but not great (fig. 1).

From this experiment we may get the impression that pre-exposure

increases both the uptake in the dark and the one in the light, but the inaccuracy of the observations with uptake in the dark does not permit us to consider it as proved. In order to get clearer data on the effects of pre-exposure we need material that possesses the substance formed

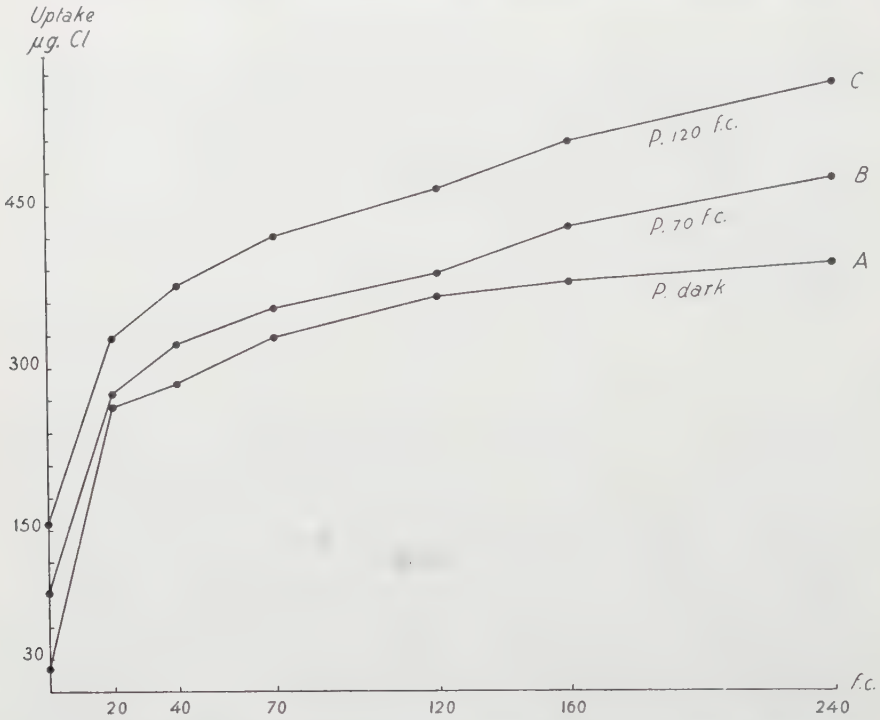

Fig. 1. Influence of exposure during the pretreatment on the uptake in different light intensities (Sodium light). Pretreatment: A. in the dark; B. in 70 f.c.; C. in 120 f.c., 24 hours. Uptake from 1 mM KCl + $CaSO_4$ solution. Aeration with air — CO_2. Abscissa light int. in foot candles. Ordinate uptake in µg Cl′ per series of 8 leafsegments (length 2.5 cm, width 0.4 cm) 25°C, 24 hours. Exp. 1098

in the light only in a slight degree before the experiment. A prolonged preceding dark period works in this direction.

In 1947 it had been investigated whether the influence of pre-exposure is only active during the first hours of uptake or that the influence of pre-exposure is perceptible during the entire uptake period. For this investigation some two experiments had been made, in which after pretreatment in the dark, at 70 f.c. and at 150 f.c. the uptake had been determined after 6 and after 24 hours (corrected statement). It was ascertained whether the effect of light during the pretreatment influenced the uptake only in the next few hours following the pre-treatment or that it lasted for a longer time. The results are given in fig. 2, which contains the data from Table 7 of Arisz's paper (1947).

It appears that through pre-exposure the rate of uptake increases in the first 6 hours as well as in the next following 18 hours. From

these experiments Arisz drew the conclusion that during the pretreatment in light a substance e.g. a sensitizer may be formed which favours the process of uptake in the light. In order to ascertain whether a sensitizer was really formed, these experiments have been repeated

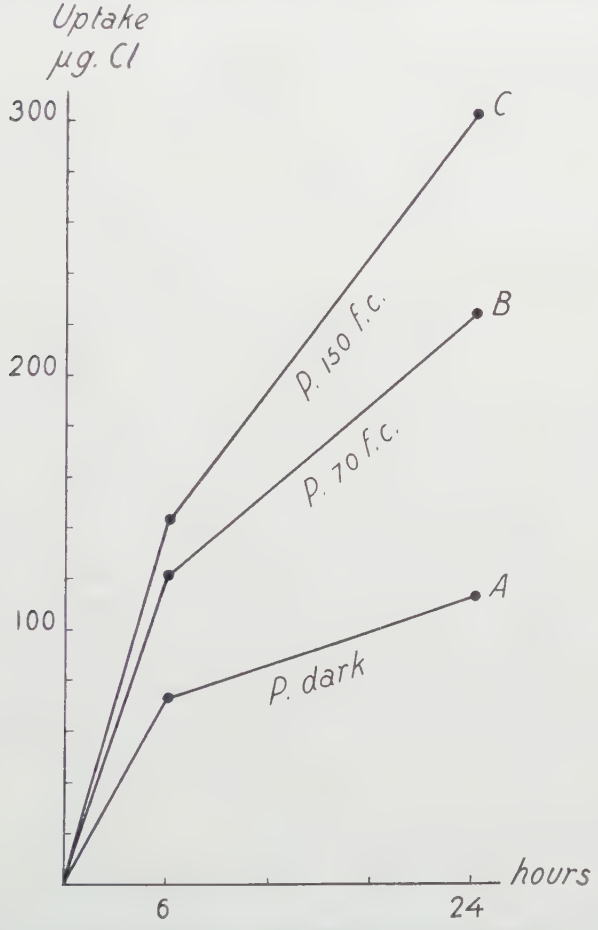

Fig. 2. Influence of exposure to light during the pretreatment on the uptake of 1 mM KCl + $CaSO_4$ solution, 24 hours, 25°C. The uptake is determined after 6 and 24 hours. A. pretreated in the dark; B. pretreated in 70 f.c.; C. pretreated in 150 f.c. Abscissa time. Ordinate uptake in µg CL'.

with strict precautions. In 1947 we had omitted to investigate whether through pre-exposure the uptake in the dark was increased as well. This is comprehensible, because the uptake in the dark for the material then cultivated in daylight was too small for such determinations to be made. In fig. 1, however, distinct differences were found for the uptake in the dark.

Fig. 3 contains the result of experiment 1107 on the uptake after

4, 8 and 24 hours in the dark and at 50 f.c., of material pretreated either in the dark or at 50 f.c. or at 150 f.c.

To obtain a clear effect the material was put in the dark for 24 hours, before the proper pretreatment was started. The result of this experiment is satisfactory. All determinations have been made in duplicate.

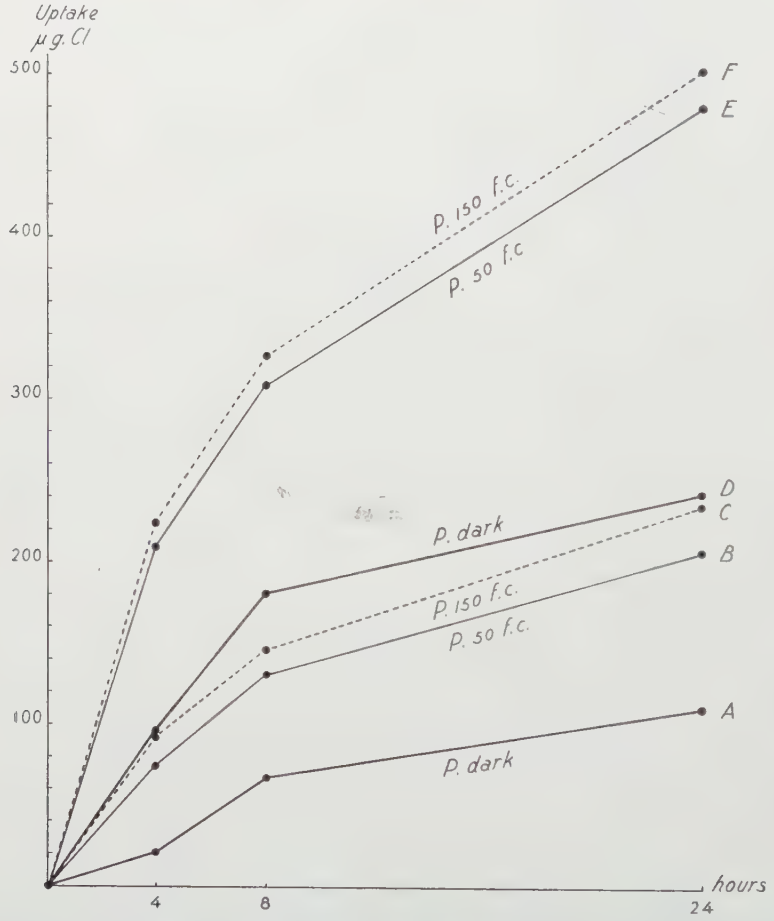

Fig. 3. Influence of exposure during the pretreatment on the course of the chloride uptake. A and D pretreatment in the dark 24 hours. B and E in 50 f.c. C and F in 150 f.c. A, B, C uptake in the dark, D, E, F, uptake in 50 f.c. 24 hours, 25°C. The uptake is determined after 4, 8 and 24 hours. Experiment 1107.

From the three lower lines A, B and C it appears that also with uptake in the dark the nature of the pretreatment has its influence. The line for pretreatment with 50 f.c. lies distinctly higher than the one for pretreatment in the dark and the one for 150 f.c. lies still higher. Besides the slope of the lines both in the first and in the second period increases distinctly after stronger pre-exposure. In the third

period the difference is still visible as well. The same thing holds good for the three upper lines D, E and F, for which the uptake at 150 f.c. was examined. Here the differences in slope between the leaflengths which have been pretreated in the dark and those exposed to 50 f.c. are much greater, but the difference with 150 f.c. pretreatment is small, because at this light intensity light saturation has been reached.

This experiment supports the previous experience that the influence of pre-exposure is demonstrable during the entire period of uptake. What is new, is that the influence of pre-exposure is also demonstrable with uptake in the dark. We must therefore assume that pre-exposure does not form a sensitizer or that the concentration of chlorophyll increases, but that a substance is formed which increases both the uptake in the dark and the uptake in the light. We may add that in this experiment too it has been our object to have exposure take place in a CO_2 free medium.

Seeing it was already demonstrated above that the substance formed in the light is transportable, it seems possible that this substance deserves the name of carrier.

We now revert to Table 15, from which it appeared that sucrose addition during pretreatment also increases the uptake. This influence of sucrose on the uptake in the light at 50 f.c. has been verified for an uptake of 4 hours, 8 hours and 24 hours (fig. 4). It appears from experiment 1106, that pretreatment with sucrose increases the uptake for the whole duration. So here too a substance is formed during the pretreatment which is itself not consumed in the uptake, so that the action diminishes, but which gives a consistently higher uptake of chloride. A few experiments originally gave us great pains with the interpretation. In these pre-exposure had but a slight effect. So in experiments 1097 and 1103 (Table 17) it makes no difference whether pretreatment takes place in the dark at 10 f.c., at 40 or at 70 f.c. An addition, however, of 1/20 M sucrose during the pretreatment distinctly raises the uptake in all cases. The effect even appears to be greater

TABLE 17

Influence of pretreatment on the uptake of chloride in the dark. Pretreatment 24 hours in the dark and in 10, 40 and 70 f.c. In A chloride uptake in the dark (24 hours), in B chloride uptake in the dark with sucrose 0.05 M, in C chloride uptake in the dark. Here sucrose was added during the pretreatment.

Exp. 1097 and 1103.

pretreatment	A uptake dark		B uptake dark with 0.05 M sucrose		C uptake dark after pretreatment with 0.05 M sucrose	
	Exp. 1097	1103	Exp. 1097	1103	Exp. 1097	1103
dark	64	32	103	156	167	209
10 f.c.	60	32	110	153	167	199
40 f.c.	57	39	103	153	167	219
70 f.c.	60	43	103	156	163	216

than, when sucrose is administered during the uptake. It is clear now that in this case material has been used which was rich in 'light substance' and poorer in 'sugar substance' so that light did not have any influence during the pretreatment and sugar did.

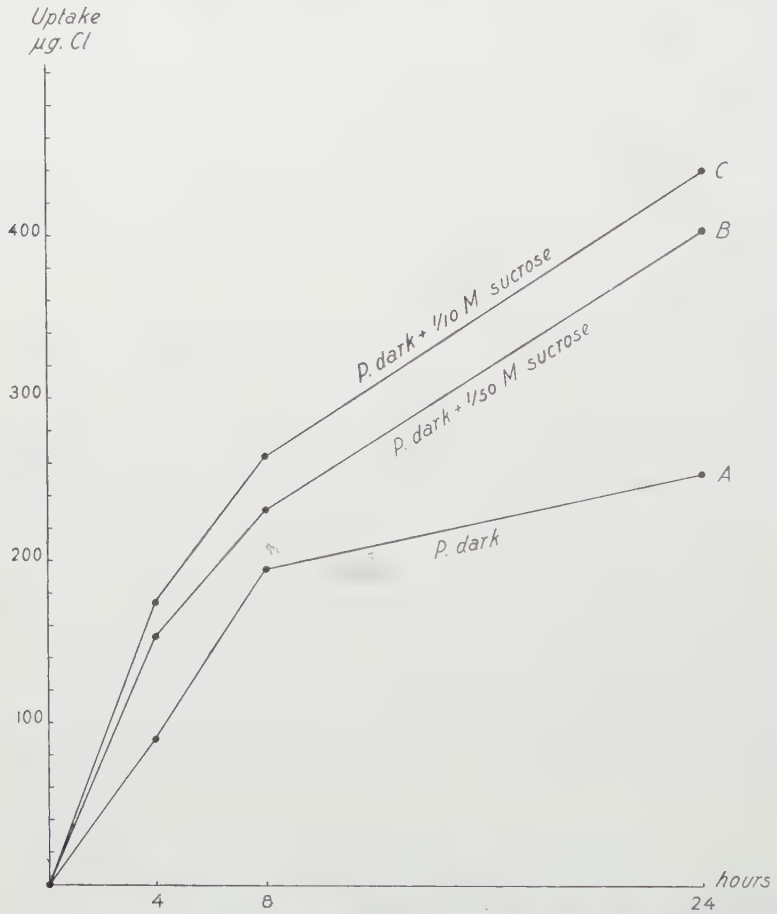

Fig. 4. Influence of supply of sucrose during the pretreatment on the course of the chloride uptake. A pretreated in the dark, B in the dark + 1/50 M sucrose, C in the dark + 1/10 M sucrose. Uptake: 50 f.c. in absence of CO_2, from 1 mM KCl + $CaSO_4$ solution, 25°C. The uptake is determined after 4, 8 and 24 hours. Experiment 1108.

In experiment 1090 (Table 18) the same was found. Here pre-exposure to 150 f.c. without CO_2 gives the same results as pretreatment in the dark, but sugar addition to the free part strengthens accumulation both in the absorbing and in the free part. These results also indicate that pre-exposure and supply with sugar during the pretreatment bring about two effects which occur independent of each other.

TABLE 18

Influence of pretreatment in the dark and in the light on the uptake of chloride from 0.01 M KCl + $CaSO_4$ added to agar strips (exp. 1090). In this case there is no difference visible.

Uptake	pretreatment in the dark	pretreatment in 150 f.c. — CO_2
in the dark	217	202
150 f.c. — CO_2	373	383
dark — dark	181 — 67 (248)	188 — 82 (270)
dark — light	245 — 153 (398)	270 — 174 (444)
dark — light + 0.05 M sucr.	323 — 227 (550)	340 — 252 (592)

Discussion

From the experiments on the influence of sucrose and light on the uptake and transport of chlorides in *Vallisneria* leaves that have been discussed here, it appears that it is possible to make considerable transports of chlorides take place and to examine them quantitatively. The great uptake in these experiments is due to the cultivation of the Vallisneria plants in artificial light in soil poor in chloride and in water from which the salt has been removed by ion exchangers. Dependent on exposure and administration of sucrose 0-500 μg Cl is taken up per series of 8 leaf segments of 2.5 cms. Most Cl is stored in the vacuoles as may be inferred from the increase of osmotic value. In the discussion of the results we have started from the symplasm theory that the ions are first taken up into the symplasm, next carried in the symplasm from cell to cell, after which there is a secretion of ions from the symplasm into the vacuoles. In *Vallisneria* leaves the mitochondria are not yet sufficiently investigated to be discussed in this paper. As carriers for intercellular transport they seem to be unsuited because they cannot migrate from cell to cell. They may have an important influence on uptake processes by providing energy or they may function as carriers in the cell cytoplasm as suggested by Robertson.

In the interpretation of the transport experiments various difficulties present themselves. It may be imagined that a transport in the leaves of Vallisneria makes use of various courses. Starting from previous results, it was found (Arisz 1947, 1948, 1953) that the symplasm is a path for transport of salts; besides we can imagine a transport through the cell walls and through the bundles in Vallisneria. It is difficult to decide whether in certain cases the cell walls act a part. In addition to the arguments already given in previous publications which point to a plasmatic transport, the influence of illuminating the absorbing zone adds a fresh argument. For does not exposure of the absorbing part render it possible that accumulation both in the absorbing and in the free zone greatly increases? As long as the absorbing zone is in the dark, the exposed free part can accumulate but small quantities of chloride. If there was a considerable uptake of chloride ions by the cells of the free part from the surrounding cell walls, the exposure of the absorbing part could not have such a great influence.

The weak transport in some experiments (e.g. experiments 1024 and 1088) shows the slight significance of the cell walls compared with the symplasm. It cannot be assumed that if the cell walls could transport ions, this process would be so variable. For a symplasm connected by the sensitive plasmodesmata, however, these differences in conductivity can be understood (ARISZ and SCHREUDER 1956). As a cause of transport in cell walls especially differences in suction tension are to be considered. In the submerged leaves of Vallisneria they do not act a part, so only diffusion in the wall capillaries would be left.

The significance of the bundles for transport will not be discussed here. We may refer to a recent paper (ARISZ and SCHREUDER 1956), from which it appears that the bundles of *Vallisneria* transport chloride, probably in a somewhat stronger degree than parenchyma cells. ARISZ (1952) considered the sieve tubes as tracts of specialised cells, which at least during part of their life transport substances in the same way as parenchyma cells do (cf. also SCHUMACHER und HÜLLSBRUCH 1955). Just as the parenchyma cells they form part of the symplasm.

We will begin with the influence of sucrose addition as it is easier to analyse than that of exposure. The sucrose is taken up into the leaf cells and converted by metabolism. Of course the supply of carbohydrates in the leaflengths with which the experiments are made, shows a great divergence owing to the different previous history and consequently a uniform reaction cannot be expected in experiments made at various times. The general impression of the influence of administering sucrose is a local stimulation of the accumulation. The effect is more localised than that of exposure, though transport of sugars certainly takes place. The favourable influence of sucrose often appears only on the spot of administration. If it is administered to the absorbing part in the dark, accumulation in the absorbing part increases, whereas it may decrease in the free part at the same time. This indicates competition of the secretion from the symplasm in the absorbing part with that in the free part. As a rule the total uptake increases in this case. By exposure and local administration of sucrose this competition may be controlled, but the unknown transport factor determining the conductivity for ions of the symplasm, will also influence the development of this competition. If, however, the absorbing zone is in the light and there are more chloride ions present in the symplasm, there is no competition since the ion supply is not limiting any more. In some cases the accumulation in the free part is then increased too.

Sucrose administered to the free part, the absorbing part being exposed to light, usually also gives a local increase of the secretion in the free part. Particularly if the absorbing zone is in the dark, the osmotic long distance phenomenon already mentioned, which can greatly reduce the accumulation in the absorbing part, appears.

This phenomenon is connected with a decrease in conductivity owing to dehydration of the symplasm (ARISZ and SCHREUDER 1956). As in the absorbing part the peripheral cells take up the chloride ions

direct from the medium and introduce them into the symplasm, the other cells will have to obtain the chloride ions through the symplasm. If, therefore the conductivity has been diminished by a suction tension, this will also make itself felt in the absorbing part. To the free part which is in touch with the sucrose and which will be able to take it up, some chloride will be carried, which may in some cases lead to considerable accumulation of chloride in the free part.

Sucrose administered during pretreatment has a stronger influence on the accumulation than if it is administered during the uptake (Table 17). It raises the rate of accumulation during several hours, during which sucrose is equally effective on uptake in the dark as in the light.

Summing up we may say that sucrose increases accumulation locally and that this may be attended by a higher uptake of ions from the medium. This must be based on a specific influence of the sugar on the absorption of ions from the outer solution or on a promotion of the secretion process, which accumulates ions from the symplasm into the vacuoles.

The local effect of sugar administration and the competitive effect between accumulation in the absorbing and the free part point to the last mentioned process.

It is conceivable that if the "membrane" as various investigators (Meyer, Teorell, Frey Wijssling, Vervelde, Arisz) assume is a Donnan system a certain relation between the chloride ion concentration in the medium and in the symplasm is being maintained, so that by diffusion of ions from the medium the concentration in the symplasm is restored, whenever it decreases by secretion of chloride ions into the vacuoles. In this way the secretion of ions into the vacuole can regulate the ion uptake from the medium. Still the rate of ion uptake into the symplasm in the dark is limited in the presence of sucrose and cannot rise above a certain level.

The influence of sugar on the uptake has been explained here by strengthening of the accumulation process, i.e. the secretion of chlorides from the symplasm into the vacuoles. We have now to consider if the symplasm of *Vallisneria* leaves is a free space in the sense of Robertson, Hope, Butler, Epstein, Burström and Lundegårdh, which is in communication with the free space in the walls and with the medium. The rate of ion uptake into the plasmatic free space must, however, be under the influence of the 'membrane', which is considered a Donnan system in this case. With regard to the symplasm we might speak of a restricted free space.

Difficulties arise, however, for the theory of free space, when the light effect is treated. For when the free part is exposed, the absorbing part being in the dark, the rate of uptake cannot exceed a certain limit.

By exposure of the absorbing part, however, the rate increases considerably, so that also the free part accumulates more chloride ions. From this it appears, as has already been discussed, that the supply of chloride ions to the free zone does not take place in the wall free space without the intermediary of the symplasm of the absorbing

zone. It also follows from this that light affects the permeation of ions into the symplasm, i.e. that under the influence of light a process takes place which causes more chloride ions to be absorbed into the symplasm. The greater availability of chloride ions in the symplasm cannot be due to the influence of light on the accumulation in the vacuole. Primary is the greater availability of chloride ions in the symplasm and secondary is the stronger secretion into the vacuoles of absorbing and free parts.

Experiments have been performed to demonstrate the presence of a free space in *Vallisneria* leaves. In Table 19 some data are given on

TABLE 19

Experiment on the presence of an apparent free space in Vallisneria leaves. After uptake of chloride from solutions of KCl of different concentrations during 24 hours the first series is analysed after rapidly rinsing in distilled water, the second series is rinsed in the dark during 15 minutes in aerated distilled water, the third series during 30 minutes. There is no loss at all of absorbed chloride ions. Experiment 1113.

	rapidly	15 minutes	30 minutes
after 24 hours' uptake of 0.00025 M KCl rinsed	259	263	266
after 24 hours' uptake of 0.001 M KCl rinsed	316	323	316
after 24 hours' uptake of 0.004 M KCl rinsed	359	362	355
after 24 hours' uptake of 0.016 M KCl rinsed	408	415	415

the uptake of different concentrations of KCl in the light and a subsequent loss of chloride ions by leakage in aerated distilled water in the dark. A loss of chloride ions from the tissue was not found. Likewise leaf segments exposed after uptake to light in distilled water during one hour showed no loss of chloride ions. Therefore the presence of a free space for chloride ions cannot be shown in this way.

Light has a strong influence on the uptake of chlorides. In the presence of a concentration of carbon dioxide higher than that of normal air a considerable quantity of carbo-hydrates is formed. It is clear that if in the photosynthesis sugars are formed, they will promote the accumulation of chlorides in the same way as has been found when sucrose was added.

Table 20 gives data about the influence of exposure to light during

TABLE 20

Influence of exposure to light during the pretreatment and the uptake in the presence or absence of carbondioxide. Light intensity 100 f.c. Uptake in μg Cl from a solution of 1/1000 M KCl + $CaSO_4$ after pretreatment: A in the light with CO_2, B in the light without CO_2, C in the dark with CO_2; duration of pretreatment and of uptake 24 hours; 25°C. Experiment S 96.

Pretreatment	uptake	μg Cl
A Light + CO_2	light + CO_2	547
	light — CO_2	523
B Light — CO_2	light + CO_2	403
	light — CO_2	410
C Dark + CO_2	light + CO_2	303
	light — CO_2	165

the pretreatment and the uptake in the presence or absence of carbon dioxide. Aeration with carbon dioxide containing air during the pretreatment has the same effect on uptake as exposure to light with addition of sugar. The experiment shows the double influence of exposure during the pretreatment, viz, the formation of a specific "light substance" which increases the following uptake and the formation of sugar in the presence of carbon dioxide. With this material exposure during uptake to light in the presence of carbon dioxide had only influence after a pretreatment in the dark.

It is evident that light also has a more specific influence on the uptake process, as both a previous exposure and an exposure during the uptake in a medium free from carbon dioxide greatly promotes the uptake. Van Lookeren Campagne (1956) found that the action spectrum of the photosynthesis in light of various wavelengths perfectly corresponds with that of the photo-accumulation of chlorides. There is a difference since the saturation for the chloride uptake was found at a lower light intensity than the saturation for photosynthesis. As presence of carbon dioxide is not required for photo-accumulation, the common feature of photosynthesis and photo-accumulation is based on the absorption and the transference of light energy by the chlorophyll. In photosynthesis this energy is used for the reduction of substances that have incorporated atmospheric carbon dioxide, in photo-accumulation for processes that cause uptake and accumulation of chlorides. It might therefore be imagined that light and sucrose would influence the uptake process in an identical way, e.g. as a result of formation of an energy rich substance both in photosynthesis and in respiration. This, however, does not seem to be the case, for even with light intensities at which uptake of chloride is maximal, a considerable increase in uptake of chloride may be caused by addition of sucrose. (Table 4). On the ground of van Lookeren Campagne's data it may be expected that the formation of an energy rich substance will be continued also at higher light intensities than at which salt uptake is maximal, so that at light saturation a dark reaction becomes limiting for the uptake. Therefore sugar cannot be active through the formation of the same substance which is formed in the light in the absence of carbon dioxide but sugar must influence the dark reaction.

Four light effects have been studied.

1. A previous exposure in carbon dioxide free medium increases the rate of chloride uptake. A substance is formed which is transportable. If an adjoining part of the exposed leaflength remains in the dark during the pretreatment it receives part of the substance formed in the exposed adjoining part, so that a subsequent chloride uptake is increased (Table 16). This substance promotes the chloride uptake as well in the dark as in the light. Therefore it is not a sensitizer but a kind of catalyst or enzyme which plays a part in the uptake. It does not seem to be readily consumed by the uptake process as it remains effective during several hours.

2. Exposure to light during the uptake in carbon dioxide free medium

promotes the uptake. The effect is dependent on the intensity of the light. The uptake may go on during many hours at the same rate or the rate may diminish after a longer or shorter time. Up to now there is no indication that during the exposure a substance is formed which increases the rate of uptake. This may indicate that exposure during uptake does not produce the substance which is formed during the pretreatment in distilled water. It seems that this substance is only formed in the light when the medium is free from salt ions. If salt ions are present the light energy is used to take the ions into the symplasm.

3. In transport experiments an exposure of the absorbing part of a 5 cm leaflength causes an increase of accumulation both in the absorbing and in the free part. If light acted only locally, as sugar does on the accumulation in the absorbing part, we should be inclined to think of an influence of light on the secretion of the ions from the symplasm into the vacuoles.

Now that, however, the accumulation in the free part also increases, even if it is in the dark, this indicates an influence of the light on the availability of ions in the symplasm. Formerly it would have been called an influence of light on the permeability and it would have been interpreted as an influence on the size of the pores in the boundary surface of the protoplasm. Nowadays, however, we know that the uptake does not only depend on the membrane but on metabolic processes as well. The data are insufficient to give a further analysis of the way light acts on the uptake process.

4. Exposure of the free zone of a transporting leaflength has an effect on the accumulation in the whole symplasm. This means that a substance is produced in the exposed free part which is transferred to the absorbing part. This would be an interesting process if we were quite sure that the substance formed in the light in the free part of the leaflength is not a carbo-hydrate. It is rather difficult to give conclusive proof that this influence on the accumulation in the absorbing part is not the result of photosynthesis in the light. Still it is remarkable that this influence is distinct even if sucrose 1/20 M is administered to both parts of the leaflengths. The experiments have therefore been repeated in carbon dioxide free medium, so that at the most the carbon dioxide produced by respiration was available to be used in photosynthesis (Table 6 exp. 1112 and Table 7A exp. 1118). It seems unlikely that this extremely small quantity of carbohydrates can have caused the rather important effect on the accumulation in the absorbing zone.

The use of longer leaflengths of 7.5 cm gives the opportunity to separate the exposed free zone from the absorbing zone by an intermediary zone of 2.5 cm, which remains in series 1 and 2 in the dark and in series 3 and 4 in the dark with addition of sucrose (Table 21). The influence of an exposure to light of the third zone in series 2 on the uptake in the absorbing zone is rather great. Addition of sucrose to the second zone (series 3) increases uptake only slightly but exposure to light of the third zone even with simultaneous addition of sucrose

to the second zone (series 4) has a considerable influence on the accumulation in the absorbing zone. This is a strong indication that light forms other substances than sucrose in the free leaf zone which are moved in the symplasm and promote the uptake and the accumulation in the absorbing part. If this conclusion is justified it raises the

TABLE 21

Influence of exposure to light of a zone of the free part on the accumulation in the absorbing zone. Leaflengths of 7.5 cm are placed in perspex boxes divided in three compartments. The first zone of 2.5 cm is exposed to light and absorbs chloride from agar strips with 0.01 M KCl, $CaSO_4$ and 0.05 M sucrose. The second zone is in the dark in distilled water, series 3 and 4 with 0.05 M sucrose added. The third zone is between agar strips containing 0.05 M sucrose. This zone is in series 1 and 3 in the dark, in series 2 and 4 exposed to light. Pretreatment in distilled water exposed to light during 24 hours. After the 24 hours' uptake the leafzones are separated and the chloride content in μg Cl of 8 single segments is estimated. Experiment 1117.

series	first zone	second zone	third zone	chloride content
1	light + sucrose —	dark	— dark + sucrose	430 — 75 — 68
2	light + sucrose —	dark	— *light* + sucrose	**543** — 110 — 117
3	light + sucrose —	dark + sucrose	— dark + sucrose	444 — 89 — 75
4	light + sucrose —	dark + sucrose	— *light* + sucrose	**529** — 124 — 110

question whether the product formed in the free part in the light in the absence of carbon dioxide could be identical with that formed during the pretreatment in the light, which as we have seen is also transportable. At the present time our knowledge of these processes is not sufficient for us to give a satisfactory answer to this question.

SUMMARY

Sucrose and light have a regulating influence on the accumulation of chloride ions in the cells of *Vallisneria* leaves. The light effect is not only the result of the formation of carbo-hydrates in the photosynthesis, light also being active in the absence of carbon dioxide. Seeing that with light saturation for the chloride uptake sucrose still increases the uptake, the influence of light and sucrose is not due to the formation of the same substance.

Light promotes the uptake into the symplasm and as a result increases the transport in the symplasm and indirectly the secretion of chloride ions from the symplasm into the vacuoles. This obtains as much for the vacuoles in the absorbing part as for those in the free part of the leaf.

If the uptake takes place in the dark, it has a limiting influence on the accumulation in the exposed free part. This proves that the free part does not take up chloride ions direct from its surroundings. Therefore transport of chlorides through the cell walls to the free part does not take place.

Exposure of the free part, the absorbing zone being exposed to light, causes an increase of accumulation in the absorbing zone. This indicates that a substance is formed by the exposed free part and transported to the absorbing part which increases accumulation of chloride ions.

Exposure of the free part, the absorbing zone being in the dark with sucrose, may likewise result in an increase of the total uptake, so that the accumulation both in the absorbing and in the free part increases. If, however, the absorbing zone is in the dark without sugar, the effect is variable.

The influence of sucrose administered to the absorbing or to the free zone is in

the main local, though sometimes an increase in accumulation occurs in the adjoining part. This can be explained by transport of the absorbed sugar.

Sucrose administered to the absorbing part in the dark increases the secretion of chloride ions into the vacuoles in the zone of administration. This sometimes takes place at the expense of the accumulation in the free part. This is due to the fact that the uptake of chloride ions in the dark and in the dark with sucrose is limited, so that the absorbing part must compete with the free part to accumulate the ions into the vacuoles. The rate of transport in the symplasm and the presence of carbo-hydrates determine the result.

Sucrose in the free zone, if the absorbing zone is in the light, likewise gives a local increase of the secretion into the vacuole; in some cases the secretion into the absorbing part also increases. Addition of sucrose to the free part may have an osmotic effect on the absorbing part, owing to which it accumulates fewer chloride ions in the vacuoles. This influence may be compensated by giving sucrose to the absorbing part.

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